STUDIES ON MAGNESIUM AMMONIUM PHOSPHATE UROLITHIASIS IN DOGS

BY

WILLIAM T. CLARK, B.V.M.S., F.R.C.V.S.

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ABSTRACT OF THESIS

Name of Candidate WM. TOWLER CLARK

Address DEPARTMENT OF VETERINARY SURGERY, UNIVERSITY OF EDINBURGH

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Title of Thesis STUDIES ON MAGNESIUM AMMONIUM PHOSPHATE UROLITHIASIS IN DOGS

This research study is essentially concerned with the etiology of magnesium ammonium phosphate urolithiasis in dogs, particularly the role of bacterial infection of the urinary tract on the formation of calculi. The problem was investigated from four aspects by carrying out: I. a clinical and bacteriological study of dogs, cats and sheep with urolithiasis; II. a study of the structure of canine calculi; III. tests on bacteria isolated from dogs with urolithiasis; IV. attempts to reproduce calculi experimentally in vitro in canine urine and in vivo in dogs.

I. Urinary tract infection in relation to urolithiasis in dogs and other animals

A review of the literature showed that other workers had noted that magnesium ammonium phosphate calculi were frequently associated with bacterial infection of the urinary tract in dogs and man. Differing opinions were expressed on infection in cats and sheep with urolithiasis. Staphylococci were recognised as the main type of organism recovered from dogs but workers studying urolithiasis in man have found a wider variety of organisms involved.

In the present study urinary tract infection was present in 82% of dogs with magnesium ammonium phosphate calculi and in 22% of dogs with calculi composed of calcium salts. Infection was absent from all dogs with cystine calculi.

In 44 cases of magnesium ammonium phosphate calculi staphylococci were isolated on 31 occasions, streptococci on 7, Proteus species on 3 and other organisms were found on 5 occasions. In 8 cases the urine was sterile.

Crystals were removed from 43 magnesium ammonium phosphate calculi and sections were prepared of the remaining organic matrices. In 41 of these sections bodies resembling bacterial cocci were present. In 33 instances these calculi were associated with staphylococcal or streptococcal infection of urine, while in the remaining cases the urine was either sterile or infected with organisms of a different type.

Dogs with urolithiasis were treated by surgical removal of calculi and antibiotic therapy. Their post-operative progress was followed by clinical and radiological examinations.

In animals with magnesium ammonium phosphate stones at the initial episode, recurrence of calculi occurred in 30% of cases while in dogs with calculi composed of calcium salts or cystine the recurrence rates were 77% and 75% respectively. At follow-up examination 11 dogs were infected with staphylococci and 9 of these had recurrent calculi composed of magnesium ammonium phosphate.

These studies confirmed the association between staphylococcal infection and magnesium ammonium phosphate urolithiasis in dogs. Bacteria did not appear to be involved in the deposition of magnesium ammonium phosphate crystals in the urinary tracts of 10 cats or 4 sheep.

II. Physical characteristics of canine urinary calculi

Calculi removed from dogs with urolithiasis were weighed and their shape recorded. Thin sections of the stones were prepared and the internal structure examined macroscopically and microradiographically. The mean weight of magnesium ammonium phosphate calculi from female dogs was 19.87g and from male animals 0.98g.

The number of magnesium ammonium phosphate calculi and their shape, size and surface texture appeared to be inter-related. Single calculi were usually of a spherical shape while large multiple calculi tended to be tetrahedra.

Examination of the internal structure of magnesium ammonium phosphate calculi

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showed that nuclei were occasionally present but did not seem to be an important cause of urolithiasis. Many of the calculi were composed of bands of crystals of alternating composition. Central fissures were also noted in several calculi.

III. Properties of bacteria associated with magnesium ammonium phosphate urolithiasis

Review of the literature showed that earlier workers considered the production of urease by bacteria and its action in raising urinary pH was important in the etiology of urolithiasis by reducing the solubility of magnesium ammonium phosphate in urine. Some bacteria can produce phosphatase and the possible effect on calculi was studied. Urease was produced by 33 out of 34 strains of staphylococci and by all 3 strains of Proteus tested. Four strains of streptococci and 2 strains of E. coli were urease negative. Phosphatase was produced by 33 out of 34 strains of staphylococci but not by the other organisms isolated from dogs with calculi. Urease and phosphatase could influence the solubility of magnesium ammonium phosphate in urine but neither enzyme was produced by all staphylococci isolated from dogs with calculi.

Tests for the production of coagulase, haemolysis and pigment production were carried out on 34 strains of staphylococci. Thirty-one strains produced coagulase, 29 strains haemolysed sheep blood, 8 strains haemolysed horse blood and 2 strains produced golden pigment.

IV. Experimental studies on urolithiasis

The literature on experimental production of calculi in vitro and in vivo was reviewed. It was found that dogs have been seldom used in experimental work.

When calculi were placed in sterile canine urine in vitro they became smaller and when placed in urine infected with staphylococci or Proteus organisms little change occurred in the calculi but there was a heavy deposit of magnesium ammonium phosphate crystals from the urine.

Experimental bladder infection with staphylococci was established in 3 dogs and 1 of these developed a magnesium ammonium phosphate calculus.

As a result of these investigations it was concluded that urinary tract infection with staphylococci is an important factor in magnesium ammonium phosphate urolithiasis. The production of urease by bacteria leads to elevation of the urinary pH and causes deposition of magnesium ammonium phosphate crystals but the mechanism by which these crystals aggregate to form calculi was not established.

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Candidate for degree of Ph.D. Faculty Veterinary Medicine.

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SUMMARY

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Examination of the internal structure of magnesium ammonium phosphate calculi showed that nuclei were occasionally present but did not seem to be an important cause of urolithiasis. Many of the calculi were composed of bands of crystals of alternating composition. Central fissures were also noted in several calculi.

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INTRODUCTORY COMMENT

Although the formation of calculi within the urinary tract of dogs has been recognised for many years as an important clinical condition and although studies by many others have established the composition of urinary calculi and have contributed information on the etiology of this condition, the disease is widely prevalent and the many problems involved in its treatment and prevention are formidable and largely unsolved.

Magnesium ammonium phosphate is the most common crystalline constituent of canine calculi. This substance also occurs in calculi which form in the other domestic species and is present in some cases of urolithiasis in man. The present study was essentially concerned with the cause of magnesium ammonium phosphate urolithiasis in dogs. Several earlier workers consider that bacterial infection is an important factor in this condition; consequently this aspect of the problem was particularly studied.

This investigation covers a series of cases studied in detail over a period of years. Many of the methods used in this investigation are common standard practice; however, techniques and research methods, some of which have proved useful in studying human urolithiasis but have not been reported as used in investigating canine calculi, have been introduced.

By collating the evidence available from a study of the literature along with the information which has resulted from the various aspects of this research study, it was hoped that further evidence might be available to help elucidate some of the mystery of this complex, intriguing and important clinical problem.

PART I Urinary tract infection in relation to urolithiasis in dogs and other animals

In this part of the project clinical cases of urinary calculi in dogs were studied with a view to obtaining information on the relationship between urinary infection and the development of urolithiasis. Cats and sheep occasionally develop urinary obstruction due to magnesium ammonium phosphate crystals so several animals of both species were examined to find out whether or not bacterial infection was present.

Several different types of calculi occur in dogs and they may be classified either by their chemical name or by their mineralogical name. Table 1 lists the crystalline constituents of canine urinary calculi.

Table 1

Crystalline constituents of canine urinary calculi

Chemical name	Mineralogical name	Chemical formula
Magnesium ammonium phosphate hexahydrate	Struvite	$MgNH_4PO_4 \cdot 6H_2O$
Apatite	Apatite	$Ca_{10}(PO_4)_6(OH)_2$
Calcium hydrogen phosphate dihydrate	Brushite	$CaHPO_4 \cdot 2H_2O$
Calcium oxalate monohydrate	Whewellite	$CaC_2O_4 \cdot H_2O$
Calcium oxalate dihydrate	Weddelite	$CaC_2O_4 \cdot 2H_2O$
Cystine		$(-SCH_2CHNH_2COOH)_2$
Ammonium acid urate		$C_5H_3N_4O_3NH_4$

1. REVIEW OF THE LITERATURE

a. A review of the literature in relation to urolithiasis in dogs

i. Incidence of different types of calculi

The incidence of the different types of canine urinary calculi in Great Britain has been studied by Hobday (1922), Goulden (1966), White (1966) and Weaver (1970). Their findings are summarised in Table 2.

Table 2

Incidence of the different types of canine urinary calculi

	Total examined	Phosphate	Oxalate	Cystine	Urate
Hobday (1922)	39	35	3	1	0
Goulden (1966)	55	23	18	13	1
White (1966)	350	211	54	67	13
Weaver (1970)	100	53	14	20	13

ii. Position of calculi in the urinary tract; sex and age distribution

Calculi were present in the renal pelves of 5% of the cases recorded by White (1966) and by Weaver (1970) while stones in the ureters have been rarely found in dogs although cases were reported by Krabbe (1949), Brodey (1955) and Finco, Kurtz and Porter (1970).

White (1966) observed that 4 out of 5 stones in the bitch were composed of phosphate while male dogs were usually affected by oxalate, cystine or urate calculi. In Weaver's series 19 out of a total of 64 male dogs had phosphate

stones but for the other types of stones and for calculi in bitches the distribution resembled White's findings.

The age of the dogs at the time of treatment was recorded by Weaver (1970) who found the mean age in years for the different type of calculi to be: phosphate 5.9, oxalate 8.5, cystine 5.3 and urate 4.7.

iii. Incidence of urinary infection with calculi

In dogs more than one type of organism may be associated with urinary infection. In order to avoid confusion, in this review, individual dogs with urolithiasis will be termed cases while each time a specific organism was isolated will be listed as one occasion.

Stainton (1922) reviewed earlier literature on urolithiasis in the dog and suggested that bacterial action on urea would make the urine more alkaline and thus promote magnesium ammonium phosphate calculus formation.

Krabbe (1949) divided the various types of calculi which occur in dogs into two categories according to whether they developed in non-infected or infected urine. In the first group she included calcium oxalate, uric acid and cystine stones, and in the second, magnesium ammonium phosphate, calcium phosphate and possibly ammonium urate.

Urinary calculi were classified as primary and secondary stones by Bloom (1954), secondary stones being defined as those which form in alkaline urine as a result of inflammation. He said that infection was important in the formation of stones composed of phosphate. Urinary tract infection was shown to be present in 80% of the cases of canine urolithiasis examined by Orstadius and Dahlberg (1966).

The types of bacteria isolated from the urine of dogs with urolithiasis were recorded by Brodey (1955), Fritsch and Zuylen (1966) and Goulden (1966).

Their findings are summarised in Table 3 (page 7).

iv. Bacteria associated with different types of calculi

The frequent association of phosphate calculus formation with alkaline urine brought about by micrococci has been noted by Milks (1935). Crabtree (1943) described a case of urinary infection in an Irish Terrier in which a urea-splitting staphylococcus was isolated. Initial radiographic examination revealed no calculus formation but two months later calculi were present.

Bacteriological examination of urine from 39 cases of phosphate calculi by Brodey (1955) yielded micrococci on 26 occasions, Proteus on 4, streptococci on 5 and coliform organisms on 7. In 5 cases the urine was sterile.

Piermattei (1960) suggested that 75% of the cases of phosphate urolithiasis in the dog are due to infection, the important bacteria being micrococci. Fritsch and Zuylen (1966) noted that in most cases where staphylococci were isolated the stones consisted of a mixture of magnesium ammonium phosphate and ammonium urate. In Goulden's (1966) study staphylococci were isolated from 13 out of 19 cases with magnesium ammonium phosphate calculi but were present in only 4 out of 36 cases with the other types of stones.

v. Recurrence of urolithiasis

Hobday (1922) expressed the view that in the majority of cases calculi do not recur but some dogs have a predisposition to stone formation and in these cases stones will return within two years. McCunn (1934) noted that stone recurrence was more frequent in the bitch and described a case where a large stone was removed and another reformed to the same size in a month. An example of very slow stone growth was published by Stainton (1922) who gave details of a Skye terrier which had a bladder calculus for over six years without causing the animal noticeable inconvenience. Its presence was confirmed by radiographic

Table 3

Bacteria isolated from urine of dogs with urolithiasis

Research worker	Number of dogs examined	Number of occasions on which each type of organism was isolated					
		Staphylococci	Streptococci	Proteus	E. coli	Other bacteria	Sterile
Brodey (1955)	46	29	5	4	7	6	11
Fritsch and Zuylen (1966)	36	17	9	5	4	7	6
Goulden (1966)	55	17	5	1	6	0	33

examination.

Brodey (1955) noted that in his series of 42 cases of phosphate urolithiasis 7 of these animals had a history of previous episodes of calculi. In a study of 133 cases Finco, Rosin and Johnson (1970) noted that 16 dogs had recurrent urolithiasis while Stockman (1972) found recurrent stones in 15 out of 23 male dogs.

Krabbe (1949) investigated the recurrence of calculi after surgical removal in a small group of dogs while Weaver (1970) studied the incidence of recurrence in 100 cases by sending questionnaires to the owners of the dogs. Weaver traced dogs for periods up to four years after the initial treatment and, although in his study all the animals were not followed for the same length of time, his results suggested that the majority of stone recurrences had taken place within two years of the initial episode. He failed to find a statistically significant difference in the recurrence of the different types of stones although he noted that there were fewer recurrences in the phosphate group.

Bacteriological tests carried out on urine from 6 cases with recurrent phosphate calculi by Brodey (1955) isolated micrococci on 5 occasions and Proteus 3 times.

vi. Leucocyte counts

Doxey (1964) found that the mean leucocyte count for healthy pet dogs over one year old was 10,137 $\pm$ S.D. 2,484 whereas Schalm (1965) studied 76 healthy dogs of all ages and found the mean leucocyte count to be 11,500 $\pm$ S.D. 2,800. Schalm also noted that in the dog infection produces a marked rise in the number of leucocytes in blood and that a similar response can be produced by a variety of non-infectious conditions which stimulate a stress reaction. He mentioned uraemia as one of these conditions.

b. A review of the literature on infection in relation to urolithiasis in cats

Concretions form fairly frequently in the urinary tracts of cats and produce two distinct patterns of clinical symptoms. The most commonly observed condition occurs in the male cat in which the urethra becomes plugged with a mass of crystalline and protein material. The other condition, which is less common, arises when larger and well defined calculi form in the bladder, producing irritation of the bladder wall. Although in both cases the mineral content is usually magnesium ammonium phosphate the concretions formed are different. It is usual to refer to the material in the former condition as a urethral plug and in the latter as a vesical calculus.

Frost (1958) recorded 6 cases of vesical calculi in cats and analysed 1 calculus which consisted of calcium and magnesium phosphate. Carbone (1965) examined urethral plugs from 29 cats by microscopic and X-ray diffraction methods and found them to be composed of magnesium ammonium phosphate.

Analysis of 20 urethral plugs and 9 vesical calculi by X-ray diffraction was carried out by Sutor, Wooley and Jackson (1970) who found that 17 plugs contained magnesium ammonium phosphate, 1 contained magnesium hydrogen phosphate trihydrate and 2 gave no pattern. The calculi contained magnesium ammonium phosphate in 7 cases, calcium oxalate monohydrate in 1 case and ammonium acid urate, surrounding a nucleus of calcium carbonate and quartz, in the remaining case.

A high incidence of infection in cases of feline urolithiasis was found by Fishler (1955), Foster (1967) and Meier (1967).

Fishler (1955) considered that infection with Proteus species was a major factor in this disease while Foster (1967) found that two thirds of his cases

had urinary infections with staphylococci, streptococci, Proteus species, Corynebacterium species, or coliform organisms.

Meier (1967) isolated Pseudomonas species from all of 11 cases which he examined. He also found Proteus species, staphylococci, Alcaligenes faecalis, Streptococcus faecalis, Escherichia coli, Aerobacter species, or paracolon organisms on some occasions.

A low incidence of infection in cases of feline urolithiasis was recorded by Rich and Kirk (1969) and by Schechter (1970).

In the series of 21 cases examined by Rich and Kirk (1969) bacteria were found in the urine from 6 animals; Str. faecalis in 3, E. coli in 1, Proteus in 1 and Staphylococcus aureus in 1 while the remaining 15 were uninfected. Urine samples from 48 male and female cats with symptoms of cystitis or urethral obstruction were studied by Schechter (1970) who found infection in only 1 case.

Rich and Kirk (1968) found that the formation of magnesium ammonium phosphate crystals in urine varied with changes in diet and urinary pH but could find no differences in crystal production between healthy cats and those developing urethral plugs. They produced obstruction experimentally in cats by introducing very large amounts of crystals into the bladder but their results would not lead them to support the hypothesis that magnesium ammonium phosphate crystals were the primary cause of urethral obstruction.

c. A review of the literature on infection in relation to urolithiasis in sheep

Three cases of magnesium ammonium phosphate calculi in sheep were recorded by Johnson, Palmer and Nelson (1940) while Beveridge (1942) in a review of the literature on urolithiasis in sheep noted that magnesium ammonium phosphate was a common constituent of these stones.

Udall and Chow (1963) regarded magnesium ammonium phosphate as the major mineral in sheep calculi and observed it as a finely divided sand-like precipitate in the bladder. They studied the effect of altering urinary pH and noted that stones formed in both acid and alkaline urine although the rate of crystal growth was probably greater in alkaline conditions.

Sutor and Wooley (1970) analysed 1 urethral calculus and 8 kidney stones by X-ray crystallography. They found that 2 of the renal calculi were composed of magnesium ammonium phosphate but the other 7 stones were non-crystalline and could not be analysed by X-ray diffraction techniques. They speculated that the material in these stones might be silica, a constituent which has also been recorded by Trueman and Stacy (1969).

The etiology of urolithiasis in sheep was discussed by Magens (1934) who considered that infection played a part in the formation of magnesium ammonium phosphate deposits. Newsom (1938) noted that urinary infection was usually present in cases of urolithiasis but he did not know whether the infection was a cause or a result of the condition. In his review of urolithiasis in sheep Beveridge (1942) concluded that urinary infection was not a primary factor in the etiology of calculi.

d. A review of the literature in relation to urolithiasis in man

i. Classification of calculi

In the majority of reports on urolithiasis in man the calculi have been classified according to their acid radical but McIntosh (1942) considered that the metallic elements were of more importance and suggested that calculi could be arranged in the following groups:-

Organic stones including uric acid, ammonium urate and cystine.

Calcium stones including calcium oxalate and calcium phosphate.

Magnesium stones including magnesium ammonium phosphate.

ii. Incidence of urinary infection with calculi

Hellström (1936) reviewed 750 cases of renal calculi and found that infection was present in 165 patients while Thompson, Steadman, Benjamin and Scott (1944) noted that renal calculi composed of magnesium ammonium phosphate were more frequently associated with infection than were the other types of calculi.

In a series of 100 cases of nephrolithiasis, Carroll and Brennan (1952a) observed that all 24 cases of magnesium ammonium phosphate stones were associated with urinary infection but only 17 of 57 cases with calcium oxalate calculi were infected.

Elliot (1968) stated that patients forming oxalate stones usually had sterile urine while those with phosphate stones had urinary infection. Hodgkinson, Peacock and Nicholson (1969) found an association between urinary tract infection and the occurrence of magnesium ammonium phosphate calculi. They also observed that the correlation of variations in the composition of the stones with sex and anatomical site appeared to be due largely to differences in the incidence of infection.

The amounts of calcium, magnesium, phosphate and oxalate excreted in urine by patients with urolithiasis was compared with the excretion of a normal group by Elliot (1968). He found no difference in the excretion of magnesium or phosphate but the amounts of calcium and oxalate were higher from patients forming calcium oxalate stones. This finding was not confirmed by Hodgkinson, Peacock and Nicholson (1969).

iii. Types of bacteria associated with calculi

Brown (1901) reported on 5 cases of calculi associated with infection. He isolated Proteus vulgaris from 3 cases and Staphylococcus albus from 2.

Staphylococci were considered by Jolly (1929) to be important in the causation of phosphate stones but Escherichia coli had little or no effect. Bugbee (1932) found that of 14 calculi containing phosphates 7 were associated with staphylococcal infection in the urine. In 13 cases of renal phosphate calculi reported by Hryntschak (1934) cocci were found in the urine on 11 occasions and E. coli isolated twice in pure culture and on 3 occasions associated with cocci.

Hellström (1956) published an extensive review of earlier work on the relationship between infection and calculus formation and particularly stressed the importance of staphylococci. He considered that Proteus could cause stone formation but streptococci and E. coli, although often associated with calculi, did not seem to be of major importance in their etiology.

Staphylococci were not found in the urine of 51 cases of phosphate urolithiasis reported by Priestley and Osterberg (1936) who observed that the organisms most commonly isolated were E. coli and Proteus species.

The ways in which bacteria might assist calculus development were considered by Keyser (1934), Hellström (1936) and Prien (1955).

Keyser (1934) observed that calculi which formed in alkaline urine were almost constantly associated with urea-splitting bacteria. From animal experiments and in vitro tests he observed that the change in urinary pH alone does not produce calculi. Keyser suggested that the organisms not only altered the pH of urine but also acted in some biologically specific manner to precipitate stone-forming crystals.

This view was largely supported by Hellström (1936). From his observation

that of 67 cases of unilateral renal calculi 23 patients had staphylococci without stones in the opposite kidney he concluded that staphylococci alone do not cause stones but require some other factor.

Prien (1955) disagreed with the suggestion that staphylococci have a special causal relationship with magnesium ammonium phosphate urolithiasis. He said that the relationship between staphylococci and calculus formation is an indirect one acting through the effect on urinary pH.

iv. Bacteria in the matrices of calculi

Bacteria were observed and cultured from the centre of a calculus by Brown (1901). Bacteriological examination of a series of stones by Eisenstaedt (1931) resulted in the isolation of staphylococci on 40 occasions, E. coli on 17, streptococci on 9 and Proteus species twice.

Hryntschak (1934) studied 43 phosphate calculi and found that 37 of them contained staphylococci. He also examined urine from his patients and noted that in some of the cases where staphylococci were present in the calculi, these organisms were not isolated from urine.

Hellström (1936) demineralised phosphate calculi by leaving them for several days in dilute hydrochloric acid. He then made smears of the organic matrices and stained them by Gram's technique. When the results of this examination were compared with the results of bacteriological tests carried out on urine from these patients, Hellström noticed that in some cases where staphylococci were seen in the stone matrices different organisms were isolated from urine.

King and Boyce (1957) measured the matrix content of human calculi and found that the average weight of matrix in calcium-containing stones was 2.5% and in cystine calculi 9% of the dry weight of the stones.

v. Recurrence of urolithiasis

Rovsing (1924) published results of nephrolithotomy on 109 human patients. He did not give the composition of the calculi but observed that recurrence occurred in 25.8% of patients with sterile urine, in 37% of patients with infection with non-urea-splitting organisms and in 79% of patients with urea-splitting bacteria.

In the published literature no long-term study of the recurrence of magnesium ammonium phosphate urolithiasis in man was found but Elliot (1968) quoted earlier investigations which showed that patients with oxalate stones and sterile urine had a recurrence rate of 29% while those with phosphate calculi associated with urea-splitting bacteria reformed calculi in 73% of the cases. Williams (1969) reviewed 538 human cases of renal calcium stones which he followed for a minimum of 10 years and a mean of 18.5 years. He found that urolithiasis recurred in 75% of the patients.

In 23 cases of recurrent phosphate stones Hellstrðm (1936) found staphylococci on 21 occasions, Proteus species and Bacillus pyocyaneus each on 1 occasion. His results are largely supported by Higgins (1939) who found infection in 81.5% of 200 recurrent renal calculi. Higgins also found staphylococci to be the main organisms isolated, Proteus being revealed less frequently. Smith (1939) however found organisms of the Proteus group to be most important in stone recurrence. Hellstrðm (1936) observed that infection with E. coli was not associated with recurrent urolithiasis.

The composition of recurrent calculi was determined by Keyser (1934) who reported that they were the same as the original stones, and this view was supported by Hodgkinson, Peacock and Nicholson (1969) who found that in 15 cases the recurrent calculi were the same as or similar to the previous stones.

Lonsdale (1968a) commented on the rate of stone growth in man and mentioned

techniques where calculi had been stained with alazarin-based dye and oxytetracycline.

e. A review of the literature on infection in relation to urolithiasis in mink

Smith and Hodson (1941) carried out chemical analysis of calculi from 11 mink and found that 10 of them were composed of magnesium ammonium phosphate. Sompolinsky (1947) noted that 0.9% of autopsies of mink in his laboratory revealed calculi in the urinary tract. In an examination of 14 stones he found the major constituent to be magnesium ammonium phosphate and suggested that urinary infection, especially during pregnancy, was an important factor in the etiology of the condition.

X-ray crystallographic analysis of mink calculi by Prien (1949) and by Nielsen (1956) confirmed that they were mainly composed of magnesium ammonium phosphate.

By carrying out bacteriological examination of urine from 84 clinically healthy animals Nielsen (1956) found that the urinary tract of mink is normally free of bacteria while examination of urine from 47 mink with urolithiasis showed that many of these were infected. He isolated micrococci on 37 occasions, while in 6 cases the urine was sterile.

Nielsen demineralised the calculi from 20 cases and prepared histological sections of the matrices. He found numerous Gram positive cocci in the matrices of 13 calculi with micrococcal infection, but no organisms were seen in the matrices of 6 cases in which the urine was not infected, nor in 1 case where Proteus was isolated.

By producing experimental micrococcal infection of the bladder in mink, Nielsen was able to induce the formation of calculi and suggested that most cases

of urolithiasis in mink originate from infection of the urinary tract with micrococci.

f. A review of the literature on infection in relation to urolithiasis in rats

Osborne, Mendel and Ferry (1917) recorded renal and vesical calculi composed of phosphate in rats fed a diet deficient in fat soluble vitamins. They suggested that as a result of the reduced resistance of the urinary tract brought about by vitamin deficiency, infection might occur, resulting in the production of ammonia which in turn could cause calculus formation. Magnesium ammonium phosphate calculi have been produced in rats by altering the vitamin A and mineral content of the diet (McCarrison, 1926; Newcomb and Ranganathan, 1929). Hedenberg (1954) studied the effects of vitamin A deficiency and urinary infection on calculus formation in rats and he decided that the concretions produced were the result of infection. He noted a low incidence of staphylococci and an absence of Proteus in the urine of these rats.

Calculi produced experimentally in male rats given oestrogen were found to consist mainly of magnesium ammonium phosphate with a certain amount of apatite (Benjamin, Wilson and Leahy, 1945). Bacteriological studies on the urine of these rats showed that all 29 animals had infection and 24 of them developed urolithiasis (Wilson, Benjamin and Leahy, 1945). E. coli was the organism most commonly isolated while streptococci and Pasteurella species were less frequently found. Wilson, Benjamin and Leahy (1945) measured the urinary pH and noted that animals in which calculi had formed had a more alkaline urine, but concluded that although infection preceded calculus formation neither the type of organism nor the ability to split urea was related to the urinary pH or the development of calculi.

Foreign bodies of various materials were introduced into the bladders of rats by Vermeulen, Grove, Goetz, Ragins and Correll (1950) in an endeavour to produce magnesium ammonium phosphate calculi. Although calculi were formed, they found that less than half of the rats developed bladder infection and they did not find a correlation between infection and calculus formation although in some instances the stones may have grown more quickly in infected cases. In a further experiment Vermeulen, Ragins, Grove and Goetz (1951) studied the effect of altering the urinary pH on stone production in rats with a zinc foreign body in the bladder and observed that reducing the pH of the urine prevented stone formation and caused dissolution of calculi which had previously formed. They again found a low incidence of infection in the rats which developed urolithiasis.

Proteus mirabilis was injected intracardially in anaesthetised rats by Braude and Siemienski (1960) and the kidneys were massaged through the abdominal wall. They found that in 13 out of 59 rats this technique produced renal calculi composed of magnesium ammonium phosphate. Calculi were not produced in uninfected control animals nor when Pseudomonas aeruginosa, E. coli or enterococci were used. Braude and Siemienski suggested that the mineral was precipitated in the alkaline environment caused by P. mirabilis.

Cotran (1963) produced renal and vesical calculi in 85% of rats with P. mirabilis infection experimentally introduced into the bladder.

2. MATERIALS AND METHODS

a. Dogs used in this study

During the period April, 1964 to June, 1968, 106 of the dogs presented for examination at the Department of Veterinary Surgery of the Royal (Dick) School of Veterinary Studies were found to be affected with urolithiasis. Sixty-five of these dogs were first examined in the Small Animal Practice Teaching Unit of the Veterinary School and as this unit referred all cases of urolithiasis to the Department of Veterinary Surgery there was therefore no deliberate selection or exclusion of cases. The remaining 41 cases were referred by veterinary surgeons in private practice and the proportion of urolithiasis cases which they referred is unknown.

Diagnosis of urolithiasis was based on case history, clinical examination and radiography of the urinary tract. A total of 110 incidents were available for study as 4 of the 106 animals presented were each treated on two occasions when different types of stones were removed.

b. Radiological examination

Radiographic techniques recommended by Douglas and Williamson (1963) were carried out using "Kodak Royal Blue" X-ray film with fast screens and either a moving or a stationary parallel grid. The focal-film distance was 91 cm and the exposure, varying according to the size of the dog, was within the range 60-65 kV and 20-40 mAS.

Radiographic examination of the kidneys was carried out with the animal in the ventro-dorsal position. The bladder and urethra were examined with the dog in the lateral position. If vesical calculi were present they were seen on the radiograph in the centre of the bladder outline.

With the exception of urate calculi, stones larger than 2 mm diameter were detected on radiographic examination as they offered considerably more resistance to the passage of X-rays than the surrounding soft structures. Urate calculi greater than 5 mm diameter could be detected but they were similar in radio-density to the surrounding tissues.

In doubtful cases different exposures were made to improve the contrast on the radiograph, the position of the dog was altered to give different projections on the film and in some dogs urine was removed from the bladder and replaced with air to increase the contrast on the film between the calculi and the surrounding structures.

On the results of radiological examinations an opinion was formed regarding the presence or absence of urolithiasis in dogs used in this research investigation.

c. Bacteriological examinations

i. Urine sampling

Urine samples for bacteriological examination were obtained at the time the animal was admitted to the Department of Veterinary Surgery hospital and before any antibiotic treatment was given. However, some animals had already received antibiotics from the veterinary surgeons who referred the cases.

The following procedure was used to obtain urine samples. The external genitalia were swabbed using an aqueous solution of 1 in 1,000 chlorhexidine ("Hibitane"), and a sterile catheter passed via the urethra to the bladder. In the male dogs a 40 cm polythene catheter was used and in female animals an 18 cm metal catheter was inserted into the urethra under direct vision using a vaginascope. The first flow of urine was allowed to escape, then a 5 ml sample was collected into a sterile universal container which was immediately sealed and stored at 4^{o}C until the bacteriological tests were carried out. According to

Vejlsgaard (1965), if urine is cooled to 4°C after collection the bacterial count does not change over a period of 5 days. In this study the majority of samples were examined within 4 hours of collection and the maximum length of storage time was 48 hours.

ii. Media

Media used for isolating bacteria from urine and for viable counts were prepared according to Cruikshank (1965). Media used for biochemical tests for characterising the organisms were made as described by Cowan and Steel (1965).

iii. Bacterial counts

Vejlsgaard (1965), reviewing a variety of techniques used to count bacteria in human urine, concluded that the surface viable count method was an accurate way of determining the number of organisms in urine.

The number of bacteria in the urine samples was determined by carrying out a surface viable count by the spreading method as described by Cruikshank (1965).

Urine dilutions of 10^{-1}, 10^{-3} and 10^{-4} were made in sterile normal saline in glass stoppered bottles using aseptic techniques. Each bottle contained glass beads and thorough mixing of the sample was ensured by vigorous shaking of each bottle 30 times. The 10^{-3} and 10^{-4} dilutions were used for counting, 0.1 ml of each being pipetted on to each of two nutrient agar and two McConkey agar plates and spread evenly over the surface of the medium with a sterile glass spreader. The undiluted urine sample was inoculated in the same way on to a nutrient agar and a McConkey plate in 0.1 ml volumes.

All the plates were allowed to dry for 15 minutes at 37°C with the lids slightly open before incubation at 37°C for 24 hours in the usual way. Plates with between 50 and 500 colonies were selected for counting. The mean counts were obtained and the number of viable organisms in the original sample

calculated. When more than 500 colonies were present they were uncountable and were recorded as more than 50 million organisms per ml of urine. If less than 50 colonies were present on the plates, those plates with the highest number of colonies were used.

Vejlsgaard (1965) concluded that organisms in numbers in excess of 100,000 per ml of urine could be regarded as indicative of infection in man; likewise Mosier (1965) considered that counts of over 100,000 organisms per ml indicated infection in dogs but counts of under 10,000 organisms per ml were probably due to contamination.

Using this criterion most of the cases in this study presented no difficulty in deciding between infection or contamination as the counts were usually in excess of 100,000 or less than 1,000 organisms per ml.

Bacteriological counts were carried out on 62 of the cases at the time of initial examination and on all the dogs at follow-up examination apart from 2 dogs which had a heavy growth of staphylococci when the urine was cultured.

iv. Isolation of bacteria from urine

Urine was inoculated on to horse blood agar, McConkey's agar and nutrient agar containing 10% sodium chloride. The urine was also inoculated into nutrient broth and all cultures incubated at 37°C for 24 hours. Direct smears made from the urine, from the broth culture and from representative colonies growing on the solid media were stained by Gram's technique.

The morphological features and the staining reactions of the bacteria together with the character of growth on culture media were used to identify the main groups of bacteria. Representative colonies from the plates were subcultured on to blood agar and nutrient broth and used for further tests to identify the species and measure some of their biochemical properties.

The organisms found in dog urine in this study grew readily on nutrient agar but McConkey's medium was also useful to identify the type of organism and to limit the swarming growth of Proteus. Staphylococci were the only organisms encountered in this study which grew on agar containing 10% sodium chloride and this medium was used to isolate staphylococci present with other organisms as a mixed infection in urine.

Detailed typing of all the organisms isolated in this study was not carried out as the intention was to identify main groups of organisms and to study staphylococci in particular.

v. Storage of organisms

Pure cultures of organisms isolated from urine samples were preserved by drying in vacuo over phosphorus pentoxide.

d. Examination of calculi

Calculi which were removed at operation were carefully washed in tap water to remove debris and blood clots, then allowed to dry at room temperature for a period of at least eight weeks. They were then weighed and samples taken as described below for crystallographic analysis by X-ray diffraction. Large stones were then cut in half; one part being placed in 10% buffered formaldehyde in saline to be used for examination of the matrix, while the other was stored in the dry state for the preparation of undemineralised sections. In cases where there were numerous small calculi whole stones were used for these two purposes.

i. Examination of the matrices of calculi

A few canine calculi were demineralised by leaving them for several days in dilute hydrochloric acid as described by Hellström (1936). Smears were made of the organic matrices and stained by Gram's technique. Bacteria could be seen

by this method but the structure of the stone was lost and it was decided to try to cut sections of the matrix with a microtome so that more information regarding the distribution of the bacteria in different layers might be obtained.

After fixation for several weeks in 10% buffered formaldehyde in saline the calculi specimens were demineralised and sections of matrix were prepared. Magnesium ammonium phosphate stones were placed in a solution of 5% formic acid in 10% formalin and ion exchange resin for 1 - 2 weeks while calcium oxalate calculi were demineralised in 5% w/v sodium ethylene diamine tetra acetate with 10% formalin for 2 - 10 weeks. Crystals were removed from cystine stones by immersion in a 0.1M solution of 5,5 diethyl barbituric acid at pH 8.6 with 10% formalin for 2 - 6 weeks. The technique used for magnesium ammonium phosphate calculi was that described by Nielsen (1956), and the procedures used for calcium oxalate and cystine stones were reported by Boyce and Garvey (1956).

When the crystals had been removed from the calculi the remaining protein matrix was dehydrated by immersion for successive periods of two hours in 50%, 70%, 96%, 100% and again 100% alcohol before being cleared in chloroform and embedded in paraffin wax. Sections were cut with a microtome at a thickness of 8 µm, then mounted on glass slides and stained with Elliot's haematoxylin and eosin (H. and E.) and also by Gram's technique using methyl violet.

The canine calculi contained a fairly small amount of matrix and after decrystallization the network of matrix collapsed very easily if it were handled excessively before being embedded in wax. The matrix also shrank to a certain extent and in some cases the outline of the original calculus was not recognisable but in most cases good sections showing the arrangement of matrix were obtained.

ii. Examination of the crystalline content of calculi

Urinary calculi contain one main crystalline compound together with smaller and variable amounts of other crystalline substances. They are therefore usually classified according to the major component. X-ray crystallographic analysis of calculi requires only very small amounts of material and this technique can also give the exact crystalline form of the substance and thus separate the various types of phosphates found in calculi. Calcium oxalate monohydrate and dihydrate can also be separated by this analysis. X-ray crystallography is a semi-quantitative technique and although the major component is easily identified, minor constituents may not be detected (Sutor, 1968).

Chemical analysis of stones is a more accurate quantitative method but the different types of phosphate calculi cannot be distinguished easily and a larger amount of material is required for the analysis.

The crystalline substances found in dog calculi have been recorded by Goulden (1966) and by Sutor and Wooley (1970). Lonsdale, Sutor and Wooley (1968) reported that on prolonged exposure to the atmosphere magnesium ammonium phosphate may change to magnesium hydrogen phosphate trihydrate.

The main crystalline constituents of the calculi studied in this project were identified by crystallographic analysis using X-ray diffraction as described by Goulden (1966).

The outer layer of large stones was used for this analysis in order to relate the composition of the most recently formed part of the stone to the bacteria present in the urinary tract at the time of examination. Samples of the dried calculus were prepared by scraping some of the outer layer of large stones or by crushing one or several small stones. Each powdered sample was mixed with a small volume of acetone then pipetted on to a glass slide where it was spread evenly and the acetone allowed to evaporate.

A monochromatic X-ray beam produced by an X-ray generator at a setting of 36 kV and 20 mA was directed to the sample on the slide and the diffracted beam collected in a scintillation counter mounted on a goniometer, which was set to rotate at a rate of 2 degrees 2θ (theta) per minute. A continuous recording from 2θ angles of 4^{o} to 45^{o} was obtained and, after passing through a discriminator unit to remove background radiation, the diffracted X-rays produced a pattern on a chart recorder. The paper trace produced by this technique consisted of a series of peaks at specific 2θ angle positions. Examples of traces obtained from canine calculi have been published by Goulden (1966). The crystal lattice spacing (d spacing) for the mineral under examination was obtained from the 2θ angle readings by reference to "Tables for Conversion of X-ray Diffraction Angles to Interplaner Spacing" (United States Department of Commerce, 1950). After obtaining the d spacing values, the mineral constituents of the calculi were identified by reference to the following publications: "Index to the X-ray Powder Data File" (American Society for Testing Materials, 1959), "A Clinical Study of Canine Urolithiasis" (Goulden, 1966) and "Identification Standards for Human Urinary Calculus Components using Crystallographic Methods" (Sutor and Scheidt, 1968).

e. Leucocyte counts

On admission to the Department of Veterinary Surgery blood samples were taken from the cephalic veins of 82 dogs, coagulation being prevented by the use of sequestrene. The samples were then diluted to a final concentration of 1 in 500 by adding 0.04 ml blood to 20 ml normal saline. From this mixture 0.2 ml was removed and replaced by 0.2 ml of 2% w/v saponin to lyse the red blood cells. The leucocytes in this sample were then counted using a Coulter Model A electronic cell counter.

f. Treatment of dogs with urolithiasis

Several owners of dogs with urinary calculi decided not to accept treatment for their animals which were duly destroyed. The remainder of the dogs were treated surgically to remove all calculi from the urinary tract. Urethral calculi were removed by urethrotomy over the site where the stones had lodged. Bladder calculi were removed by laparotomy and by cystotomy, incising the dorsal aspect of the bladder. The result of the surgical treatment to remove all the calculi was checked by radiological examination a few days after operation and if any stones remained a further operation was carried out to remove them.

In cases where bacteria were isolated from the urine the antibiotic sensitivities of the organisms were determined and treatment with appropriate antibiotics given parenterally for several days. The effectiveness of antibiotic treatment in clearing urinary infection was checked by culture of post-treatment urine samples. In most cases the urine was sterile at the end of the treatment period but in a few animals infection persisted.

g. Follow-up of dogs with urolithiasis

In order to determine the proportion of animals which developed further calculi and to obtain information on their rate of development, the owners of treated dogs were requested to bring their animals back for radiological examination of the urinary tract and bacteriological examination of a urine sample. It was decided to examine all the dogs surviving the initial episode at intervals of 12 and 24 months after treatment. Although difficulty was experienced in contacting some of the owners and many failed to bring their animals at the arranged time, most owners eventually co-operated and radiographs and urine samples were obtained.

The number of cases re-examined was reduced for a number of reasons. Some dogs were destroyed without treatment, some died at the time of surgery or within a month of the initial episode, while others survived surgical treatment but died over a month later. In the last circumstance careful enquiry was made as to the cause of death and in no case did it appear that calculi had recurred and so contributed to the death of the animal. Some dogs were lost to the study because the owners could not be traced or in a few cases they refused or were unable to co-operate further.

Animals were classified as free of recurrence if radiological examination for urolithiasis was negative at a minimum period of 21 months after the initial episode.

h. Examinations carried out on cats with urolithiasis

Radiographs of the urinary tract were taken before treatment. Urine samples were obtained by urethral catheterisation and examined for bacteria by the techniques already mentioned (page 20). Vesical calculi or urethral plugs were obtained from 10 cases and examined by X-ray crystallography as described earlier (page 25).

i. Examinations carried out on sheep with urolithiasis

Radiological examination of the urinary tract was not carried out as wool and rumen contents cause so much scattering of the X-ray beam that the distribution of calculi in the urinary tract could not be recorded. Urine samples were obtained from urine passed per urethram or by paracentesis of the bladder through the abdominal wall. These samples were immediately submitted for microscopic and cultural examination for bacteria by the Department of Veterinary Pathology. In 7 sheep crystalline material was removed from the urethra and analysed by X-ray crystallography.

j. Statistical calculations

In this study the results obtained were subjected to statistical analysis using tests described by Fisher (1970), Hill (1966) and Snedecor (1956). Statistical tables published in "Geigy Scientific Tables", Geigy (1970), were used.

3. RESULTS

a. Dogs used in this study

The breed, age and sex of the 106 dogs studied in this project, together with the chemical composition of the calculi removed from each animal, are detailed in Appendix 1.

Four dogs had recurrent calculi differing in composition from the original stones. In each case the recurrent calculi were composed of magnesium ammonium phosphate and the episodes are designated 41A, 42A, 43A and 44A. In cases 43 and 44 the initial stones were composed of calcium oxalate while in cases 41 and 42 cystine calculi were removed at the initial episode.

b. Incidence of different types of calculi in dogs

The calculi were classified according to their main crystalline component. In most earlier studies on canine urolithiasis the investigators have divided the stones into four groups, namely: phosphate, oxalate, cystine and urate, and on this basis the distribution for the present series is given in Table 4 with the results of studies by Weaver (1970) and White (1966).

Table 4

Incidence of different types of calculi

	Total examined	Phosphate	Oxalate	Cystine	Urate
This Series	110	49 (44%)	35 (32%)	24 (22%)	2 (2%)
Weaver (1970)	100	53 (53%)	14 (14%)	20 (20%)	13 (13%)
White (1966)	350	211 (60%)	54 (16%)	67 (19%)	18 (5%)

Statistical analysis confirmed that there was a difference between the distribution of stone types in this series and the cases reported by Weaver (1970) (chi-square = 17.44, d.f. 3, $p<0.001$) and also between this series and the stones analysed by White (1966) (chi-square = 17.66, d.f. 3, $p<0.001$). When White's results were compared with Weaver's results there was also a significant difference in the distribution of the various types of stone (chi-square = 7.82, d.f. 3, $p<0.05$).

The higher incidence of oxalate stones in the present series was significantly different from Weaver's results (chi-square = 7.1, d.f. 1, $p<0.01$) and from White's series (chi-square = 11.6, d.f. 1, $p<0.001$). There were significantly less urate stones in this series than in Weaver's series (chi-square = 9.3, d.f. 1, $p<0.01$). The incidence of the other types of calculi did not differ significantly.

The above classification groups together three different types of phosphate stone namely magnesium ammonium phosphate hexahydrate, calcium hydrogen phosphate dihydrate and apatite and if these are listed separately the present series would comprise:

Magnesium ammonium phosphate	45	Calcium oxalate monohydrate and dihydrate	35
Calcium hydrogen phosphate	1	Cystine	24
Apatite	3	Ammonium acid urate	2

c. Position of calculi in the urinary tract

All the dogs had calculi either in the bladder or urethra with the exception of cases 104, 105 and 106 where calculi were present only in the renal pelvis and case 61 where calculi were found in the renal pelvis, ureter, bladder and urethra. Fig. 1 shows these calculi in the urinary tract of case 61 on a

radiograph taken at autopsy.

d. Cases excluded from further study

It was decided to confine this study to bladder and urethral stones and cases 104, 105 and 106 were excluded on these grounds. These cases had other unusual features. Cases 104 and 105 were associated with widespread bone demineralisation and abnormalities of serum calcium and phosphate levels which were thought to be due to a parathyroid gland disorder. Case 106 was found to have had a bladder tumour 13 months before the renal stone developed and at autopsy a papillary adenocarcinoma was found to be partially occluding the left ureter. An organism of the Proteus group was isolated from the urine in this case and the main mineral element in the stone was magnesium ammonium phosphate. Cases 102 and 103 developed ammonium urate stones but as they formed such a small group and as infection was absent in both cases it was decided not to study them further. After these exclusions, 105 stone incidents involving 101 dogs remained.

e. Classification of calculi

McIntosh (1942) suggested that stones could be better classified according to their metallic element rather than their acid radical and suggested three main groups - magnesium stones, calcium stones and organic stones.

Some evidence in support of McIntosh's classification was obtained when the recurrent calcium oxalate, calcium phosphate and apatite stones were studied. On three occasions a change from one type to the other occurred although there had been no known alteration in circumstances to account for the change in the acid radical (Table 5).

Table 5

Alteration of the acid radical in calcium calculi

Case	1st Episode	2nd Episode	3rd Episode
60	Calcium oxalate	Calcium hydrogen phosphate	
61	Calcium hydrogen phosphate	Calcium oxalate	Calcium hydrogen phosphate
79	Apatite	Calcium oxalate	

The 105 episodes of urolithiasis studied in this series of 101 dogs are reclassified in Table 6.

Table 6

Calculi used in this study

Type of calculus	Number of episodes
Magnesium ammonium phosphate	44
Calcium	37
Cystine	24
Total	105

f. Sex distribution of dogs with urolithiasis

The sex of all the animals is given in Appendix 1 and summarised in Table 7.

Table 7

Sex distribution of dogs with urolithiasis

Type of calculus	Male	Female
Magnesium ammonium phosphate	11 (25%)	33 (75%)
Calcium	33 (89%)	4 (11%)
Cystine	24 (100%)	0 (0%)
Total	68 (65%)	37 (35%)

g. Age distribution of dogs with urolithiasis

The age of all the animals is given in detail in Appendix 1. The age was calculated on an age last birthday basis and the true age at treatment should, on average, be six months longer. The mean age at treatment for the different groups of dogs is summarised in Table 8.

Table 8

Mean ages of the dogs at the time of treatment

Type of calculus	No. of animals	Mean age (years)	S.D.
Magnesium ammonium phosphate	44	6.0	± 2.88
Calcium	37	8.6	± 3.09
Cystine	24	4.9	± 2.03

Statistical analysis of these results showed a significant difference between the mean ages for magnesium ammonium phosphate and calcium calculi ($t = 12.4$, d.f. 79, $p<0.001$), magnesium ammonium phosphate and cystine calculi ($t = 5.2$, d.f. 66, $p<0.001$) and between calcium and cystine calculi ($t = 5.3$, d.f. 59, $p<0.001$).

h. Incidence of urinary infection with different types of calculi in dogs

The detailed results of bacteriological examination of the urine are given in Appendix 2. The overall rate of infection for the three groups studied is recorded in Table 9.

Table 9

Incidence of urinary infection with different types of calculi

Type of calculus	Infected	Sterile
Magnesium ammonium phosphate	36 (82%)	8 (18%)
Calcium	8 (22%)	29 (78%)
Cystine	0 (0%)	24 (100%)
Total	44 (42%)	61 (58%)

Statistical analysis of these results showed that infection is more commonly found with magnesium ammonium phosphate calculi than with calcium calculi (chi-square = 29.8, d.f. 1, $p<0.001$) and that it is also more frequently associated with magnesium ammonium phosphate than with cystine calculi (chi-square = 38.5, d.f. 1, $p<0.001$).

The relationship between urinary infection and the sex of the animal is given for cases with magnesium ammonium phosphate calculi in Table 10.

Table 10

Relationship between urinary infection and sex in cases with magnesium ammonium phosphate calculi

Sex	Infected	Sterile
Male	9	2
Female	27	6

In both sexes the same proportion of dogs had urinary infection.

i. Bacteria associated with different types of calculi in dogs

Smears of canine urine showing bacteria associated with urolithiasis are illustrated in Figs. 2 - 4. The number of occasions on which each type of organism was isolated is given in Table 11.

Table 11

Bacteria associated with different types of calculi in dogs

Type of organism	Type of calculus	No. of occasions isolated	Pure culture	Mixed infection
Staphylococci	Magnesium ammonium phosphate	31	23	8
	Calcium	4	2	2
	Cystine	0	0	0
	Total	35	25	10
Streptococci	Magnesium ammonium phosphate	7	2	5
	Calcium	3	0	3
	Cystine	0	0	0
	Total	10	2	8
E. coli and coliform organisms	Magnesium ammonium phosphate	5	1	4
	Calcium	4	3	1
	Cystine	0	0	0
	Total	9	4	5
Proteus	Magnesium ammonium phosphate	3	1	2
	Calcium	0	0	0
	Cystine	0	0	0
	Total	3	1	2
	Overall total	57	32	25

In these results the highest incidence of infection was due to staphylococci associated with magnesium ammonium phosphate stones. The incidence of this type of infection in the different groups of stones is compared in Table 12.

Table 12

Incidence of staphylococcal infection associated with different types of calculi

	Staphylococci isolated	No Staphylococci isolated
Magnesium ammonium phosphate	31 (70.5%)	13 (29.5%)
Calcium	4 (10.8%)	33 (89.2%)
Cystine	0 (0.0%)	24 (100.0%)

Statistical analysis confirmed that staphylococcal infection was more commonly associated with magnesium ammonium phosphate calculi than with either calcium stones (chi-square = 26.8, d.f. 1, $p<0.001$) or cystine stones (chi-square = 28.3, d.f. 1, $p<0.001$).

j. Bacteria in the matrices of calculi from dogs

Crystals were removed from 3 magnesium ammonium phosphate calculi by immersion in dilute hydrochloric acid. Smears of the matrices were stained by Gram's technique and numerous cocci were present (Fig. 5).

Sections of the matrices of all the magnesium ammonium phosphate calculi were examined with the exception of 4 cases where there was no material left after X-ray diffraction analysis and 2 cases where, on demineralisation, the stone disappeared completely. Also examined were 5 calculi from recurrent

cases plus 5 calcium oxalate and 2 cystine stones. Figs. 6 - 9 show examples of matrix sections.

The detailed findings in the 50 calculi examined is given in Appendix 3 together with a summary of the bacteria found in the urine at the time of removal of the stones. The recurrent calculi are identified by the letter R after the case number, i.e. cases 10R, 18R, 33R, 38R and 41AR.

The main finding in this part of the investigation was that a high proportion (41 out of 43 cases - 96.5%) of magnesium ammonium phosphate calculi contained numerous round bodies morphologically similar to bacterial cocci.

With Gram's stain some cocci were stained blue (Gram positive) and others red (Gram negative), most sections containing both Gram positive and Gram negative cocci. One part of the stone was usually predominantly positive and another part negative and in some sections there were areas where no bacteria were seen. Details of the distribution of the cocci in the stones are given in Appendix 3. Several sections were very small and some had broken when the section was being cut but, in the intact sections, the distribution of cocci in the central and peripheral parts of the stone was noted. When sections of magnesium ammonium phosphate calculi were stained by Gram's technique, the protein matrix stained pink or red and was arranged in long strands often grouped together and sometimes with a concentric formation. The amount and density of staining of this matrix varied considerably in different calculi.

When the sections were stained by H. and E. the cocci were stained blue while the matrix was more clearly seen stained either red or blue.

In 7 cases magnesium ammonium phosphate calculi were associated with Gram negative bacteria (Proteus species, E. coli or coliform organisms) in the urine at the time of removal of the stones, but in no case were Gram negative organisms seen

in sections of these calculi. Infection of the urine with cocci (staphylococci or streptococci) was found in 33 instances while 41 stones contained cocci when sections were examined.

Two calculi from one dog (cases 38 and 38R) showed no evidence of bacteria although the matrix was clearly seen. The urine from this case was sterile on original examination and sterile when examined at the time of stone recurrence. These calculi differed in appearance from other magnesium ammonium phosphate calculi as they were made up of large flat plates rather like some types of calcium oxalate stones. On X-ray crystallographic analysis, tracings typical of magnesium ammonium phosphate with a little calcium oxalate were obtained and this result was confirmed by quantitative chemical analysis carried out by Dr. H. Paver, Department of Animal Health, University of Edinburgh, who found:-

Calculus 38 - Magnesium ammonium phosphate 86.0%, calcium 1.4%

Calculus 38R- Magnesium ammonium phosphate 92.7%, calcium 2.1%

There were no cases of magnesium ammonium phosphate urolithiasis where cocci were present in the urine but absent in the matrix and with regard to the presence or absence of cocci the results of the bacteriological examination of the urine corresponded with the histological examination of the calculi in 35 out of 43 episodes (81%). Statistical analysis of these results confirmed that cocci were more likely to be detected by examining the stone than by examining urine. (chi-square = 4.7, d.f. 1, $p < 0.05$).

The calcium oxalate and cystine calculi had matrices which resembled those found in magnesium ammonium phosphate stones but no bacteria were seen in these calculi although in two cases the calculi were present in urine infected with staphylococci.

k. Recurrence of urolithiasis in dogs

The fate of the dogs treated for urolithiasis and the numbers of cases available for follow-up study are summarised in Table 13.

Table 13

Fate of dogs treated for urolithiasis

Outcome of case	Type of calculus			Total
	Magnesium ammonium phosphate	Calcium	Cystine	
No treatment or died during treatment	7	5	10	22
Died from causes other than urolithiasis	5	6	3	14
Lost	2	4	3	9
Available for follow-up study	30	22	8	60

The case details of all dogs available for follow-up study are given in Appendix 2 which includes information about the time which had elapsed between the first episode and the subsequent examination, and the results of bacteriological examination of the urine at this latter time.

The overall recurrence rate and the recurrence rates for different types of calculi are given in Table 14.

Table 14

Number of cases of urolithiasis showing recurrence of calculi

Initial calculus	Recurrence	No recurrence
Magnesium ammonium phosphate	9 (30%)	21 (70%)
Calcium	17 (77%)	5 (23%)
Cystine	6 (75%)	2 (25%)
All types	32 (53%)	28 (47%)

Statistical analysis of the difference in recurrence rates between magnesium ammonium phosphate calculi and calcium calculi showed the higher recurrence rate in the latter to be significant (chi-square = 9.5, d.f. 1, $p < 0.01$). The figures are insufficient to test whether there is a statistically significant difference between the recurrence rates for magnesium ammonium phosphate and cystine calculi, but when the cystine stones are added to the calcium group the difference between the recurrence rates for magnesium ammonium phosphate and the combined calcium and cystine calculi was also significant (chi-square = 11.3, d.f. 1, $p < 0.001$).

1. Length of follow-up time

In order to check that the difference in recurrence rates between magnesium ammonium phosphate and calcium and cystine stones was not due to a difference in the follow-up time, the mean time, in months, was calculated for the cases without recurrence (i.e. those followed for at least 21 months without evidence of reforming stones). The results of this calculation are given in Table 15.

Table 15

Mean follow-up time in months for cases without recurrence

Type of calculus	Number of cases	Mean time in months	S.D.
Magnesium ammonium phosphate	21	26.5	± 3.1
Calcium and cystine	7	27.4	± 2.2

Statistical analysis of these results revealed no significant difference in the follow-up time of these groups ($t = 0.56$, d.f. 26, $p > 0.05$).

m. Bacteria associated with recurrent calculi in dogs

A summary of the overall relationship between recurrence and infection is given in Table 16. Cases which showed no recurrence and were followed for less than 21 months were not included.

Table 16

Incidence of infection and calculus recurrence at the last examination

Initial calculus		Infected		Sterile	
Magnesium ammonium phosphate	Recurrence	6		3	
	No recurrence	4		17	
Calcium	Recurrence	2		15	
	No recurrence	2		3	
Cystine	Recurrence	2		4	
	No recurrence	1		1	
Overall	Recurrence	10	(31%)	22	(69%)
	No recurrence	7	(25%)	21	(75%)
Infection at initial episode		44	(42%)	61	(58%)

The incidence of infection in recurrent cases was compared with the incidence of infection at the initial episodes in the whole series of 105 cases. Statistical analysis showed that there was no significant difference between the rates of infection in these series (chi-square = 0.77, d.f. 1, $p > 0.05$).

When the results were divided according to the type of stone removed at the initial episode, it was found that recurrence was likely to be associated with infection in the magnesium ammonium phosphate group while recurrent calculi in the calcium and cystine groups were not usually associated with infection. The figures were inadequate for statistical analysis but resembled the results obtained for these groups at the initial episodes.

Staphylococci were isolated from 9 of the 10 cases of recurrence associated with infection while in the remaining case Proteus was isolated. The rate of stone recurrence in 11 dogs with staphylococci at follow-up (minimum follow-up time 21 months if no recurrence) is given in Table 17.

Table 17

Recurrence of calculi in dogs with staphylococci at follow-up examination

Initial calculus	Recurrence	No recurrence
Magnesium ammonium phosphate	5	2
Calcium	2	0
Cystine	2	0
Total	9	2

In Table 18 the relationship between staphylococcal infection and stone recurrence is given for dogs which had magnesium ammonium phosphate calculi at the initial episodes.

Table 18

Incidence of staphylococcal infection in recurrent calculus formation in dogs which had magnesium ammonium phosphate at the initial episode

	Staphylococci	No Staphylococci
Recurrence	5	4
No recurrence	2	19

Recurrence occurred in a higher proportion of the animals with staphylococcal infection than those without but the figures are inadequate for statistical analysis.

n. Composition of recurrent calculi in dogs

The type of calculus at the time of the initial episode is given in Table 19 and related to the type of stone at the recurrent episode. The results are incomplete because some dogs showed no clinical abnormality although there was clear radiological evidence of stone recurrence, and operations to remove these calculi were not justified; consequently their composition was not determined.

Table 19

Composition of initial calculi compared with recurrent calculi

Initial calculi	Recurrent calculi			
	Magnesium ammonium phosphate	Calcium	Cystine	Not determined
Magnesium ammonium phosphate	5	0	1	3
Calcium	2	5	0	10
Cystine	2	0	4	0

In 14 out of 19 recurrent calculi examined it was found that they had the same composition as the calculi removed at the initial episodes. The single case in which the dog developed a cystine stone on recurrence after showing a magnesium ammonium phosphate calculus (case 42A) had had a cystine stone removed on a prior occasion (case 42).

Four of the cases (41, 42, 43 and 44) which had initially either calcium or cystine calculi developed recurrent magnesium ammonium phosphate stones and all had staphylococci present in the urine at the time of recurrence.

Further observations were made on two dogs which had cystine calculi in the first instance. Case 90 had sterile urine at the time of the first episode but during treatment developed bladder infection with Proteus which persistently resisted treatment. This dog had an elevated urinary pH and was destroyed four years subsequent to the calculus incident. On removal of the urinary tract at autopsy small deposits of cystine were found in the kidneys but no calculi were found in other parts of the urinary tract (Fig. 10).

Case 92 suffered a recurrence of cystine calculi and was then given sodium bicarbonate by mouth to make the urine constantly alkaline (Treacher, 1966). The dog remained free from calculi for four years after this recurrence.

o. Treatment of recurrent magnesium ammonium phosphate calculi in dogs

When magnesium ammonium phosphate calculi reformed in the presence of staphylococcal infection it was considered that this might be due to inadequate treatment of the infection and therefore in two cases further surgical and antibiotic treatment was carried out. The results of these cases are given in Table 20.

Table 20

Recurrence of magnesium ammonium phosphate calculi in two cases following treatment

Case	Initial episode	1st Recurrence		2nd Recurrence		Last check	
	Bacteria	Time (months)	Bacteria	Time (months)	Bacteria	Time (months)	Bacteria
33	Staph.	3	Staph.	-		26	Coliforms No recurrence
41A	Staph.	4	Staph.	4	Staph.	21	Sterile No recurrence

p. Rate of development of recurrent calculi in dogs

All the dogs were examined radiologically at intervals after the initial treatment and the times and results of these examinations in the dogs which eventually developed recurrent stones are given in Table 21 (page 49).

There was considerable variation in the times of repeat examinations because some dogs with recurrence developed clinical signs and were examined

forthwith, while in others there were delays in getting the owners to bring their dogs. The results are divided up into three groups - less than 1 year, 1 - 2 years and over 2 years.

An example of a case involving rapidly growing recurrent magnesium ammonium phosphate calculi is shown in Figs. 11 - 14.

Table 21

Time taken for calculi to reform

Case no.	Time of examination after initial episode			Composition of recurrent calculus
	Less than 1 year	1 - 2 years	Over 2 years	
2	+			Unknown
10		+		Mg.Am.Phos.
11	+			Unknown
16	+			Unknown
18	+			Mg.Am.Phos.
33	+			Mg.Am.Phos.
38	+			Mg.Am.Phos.
41A	+			Mg.Am.Phos.
42A	-	-	+	Cystine
43	+			Mg.Am.Phos.
44		+		Mg.Am.Phos.
50	+			Unknown
51	-	+		Unknown
52	+			Unknown
53		+		Unknown
55		+		Ca.Ox.
58	+			Unknown
60	+			Ca.H.Phos.
61	+			Ca.Ox.
65	-		+	Unknown
66	+			Unknown
67		+		Ca.Ox.
69	+			Unknown
74		+		Unknown
78		+		Unknown
79	-		+	Ca.Ox.
41	+			Mg.Am.Phos.
42	+			Mg.Am.Phos.
85	+			Cystine
92	+			Cystine
93	+			Cystine
94	+			Cystine

\+ Radiological evidence of recurring calculi

\- No radiological evidence of recurring calculi

q. Leucocyte counts in dogs

The leucocyte counts in blood samples from 82 clinical cases of urolithiasis is given in Appendix 4. The cases in which calculi were obstructing the free passage of urine are also indicated in this appendix.

These results are summarised in Table 22 which gives mean values for cases with and without urinary infection and also compares the counts in animals which had urinary obstruction with those free of obstruction.

Table 22

The relationship of the mean leucocyte counts to urinary infection and urinary obstruction

Type of case	No. of cases	Leucocyte count	S.D.
Infected	35	14,674	± 712
Sterile	47	13,563	± 745
Obstructed	43	15,067	± 937
Non-obstructed	39	12,066	± 765
Infected and obstructed	8	17,150	± 513
Sterile and non-obstructed	12	10,750	± 411

Statistical analysis of these results showed that there was a significant difference between the leucocyte counts from dogs with urinary infection and those with sterile urine ($t = 6.9$, d.f. 80, $p < 0.001$). There was also a significant difference in leucocyte counts between dogs which had urinary obstruction and those free of obstruction ($t = 15.5$, d.f. 80, $p < 0.001$).

The greatest difference in white cell counts was found when the mean

count from dogs with both infection and obstruction was compared with the mean value from animals with neither infection nor obstruction. The difference was again significant ($t = 30.9$, d.f. 18, $p < 0.001$).

r. Infection and urolithiasis in cats

The detailed results of the examinations carried out on cats are given in Table 23. Urinary infection was found in only one animal affected with urolithiasis.

Table 23

Clinical cases of urolithiasis in cats

Age	Sex	Site	Bacteriology	Calculus analysis
12y	Mc	Bladder and urethra	Sterile	Mg.Am.Phos.
5y	Mc	Bladder	Sterile	Mg.Am.Phos.
8y	Mc	Urethra	Sterile	Mg.Am.Phos.
2y	M	Urethra	Sterile	Mg.Am.Phos.
13y	Mc	Urethra	Sterile	Mg.Am.Phos.
1y	M	Urethra	E. coli	Mg.Am.Phos.
5y	Mc	Urethra	Sterile	Mg.Am.Phos.
2y	Mc	Urethra	Sterile	Mg.Am.Phos.
12y	Mc	Urethra	Sterile	Mg.Am.Phos.
3y	Mc	Urethra	Sterile	Mg.Am.Phos.

M = male Mc = male castrated

s. Infection and urolithiasis in sheep

The detailed results of the examinations carried out on sheep are given in Table 24. Urinary infection was present in 3 out of 7 sheep with urolithiasis.

Table 24

Clinical cases of urolithiasis in sheep

Breed	Age	Sex	Site	Bacteriology	Calculus analysis
Blackface	1y	M	Urethra	Sterile	Mg.Am.Phos.
Blackface	n.k.	M	Urethra	Cl. welchii	Mg.Am.Phos.
Blackface	1y	M	Urethra	Sterile	Mg.Am.Phos.
Down x	2m	M	Urethra	P. mirabilis	Mg.Am.Phos.
Blackface	10m	M	Urethra	E. coli	No pattern
Cheviot x	1m	M	Urethra	Sterile	No pattern
Cheviot	2y	M	Urethra	Sterile	No pattern

n.k. = not known

Three calculi samples could not be identified by X-ray crystallography as they produced no pattern and were probably non-crystalline. Insufficient material was available for chemical analysis of the calculi from these cases.

4. DISCUSSION

a. Incidence of different types of calculi in dogs

There was a higher incidence of oxalate calculi and fewer urate stones in this series than in those reported by Weaver (1970) and White (1966). Phosphate calculi however were the type most frequently found in all three series although they formed less than 50% of the cases in the present study. The variations in incidence between these series of calculi can probably be explained by the different sources of the stones. White's calculi were sent to him for analysis by veterinary surgeons and it is likely that there was some selection of the specimens they chose to send. Weaver's dogs were all referred for treatment by veterinary surgeons in practice and again some degree of selection may have taken place. In both these series phosphate calculi might be over-represented as they are usually larger than the other types.

Genetic factors are thought to play a part in cystine stone formation (Brand, Cahill and Kassell, 1940) and excess excretion of urate is an inherited defect in Dalmatians, so that variations in breed distribution may account for part of the difference in incidence of the different types of calculi in these three series.

The distribution of the different types of calculi in the present series closely resembles the results obtained by Goulden (1966). The clinical records of 47 cases which Goulden used in his series have been included in the present study and extending the series over a greater number of cases has largely confirmed his findings on distribution.

b. Position of calculi in the urinary tract

Weaver (1970) and White (1966) noted that few calculi occur in the renal pelvis in the dog and this observation was confirmed in the present series where

renal calculi were found in only four dogs. Calculi in the ureter have been recorded only rarely in dogs (Krabbe, 1949; Brodey, 1955; Finco, Kurtz and Porter, 1970) but one case was found in the present series where a calculus was obstructing a ureter. Over 95% of the cases had calculi in the bladder or in the urethra or in both these organs and it was decided to limit this study to dogs with urolithiasis at these sites.

c. Classification of calculi

Excluding renal and urate calculi the remaining stones were divided according to McIntosh's (1942) suggestion into groups in which the main crystalline constituent contained either magnesium or calcium or cystine.

The etiology of calcium-containing stones is unknown apart from the small number of cases where there is dysfunction of the parathyroid gland. Calcium calculi are more frequently found in man than in the dog. In man the disease has been extensively studied but there is disagreement as to whether patients forming calculi excrete excessive amounts of calcium and oxalate (Elliot, 1968; Hodgkinson, Peacock and Nicholson, 1969). The urinary excretion of calcium and oxalate has not been extensively studied in the dog although Goulden (1969) found no difference between the serum levels of calcium and phosphate in dogs with calcium calculi and those with other types of stones.

Two dogs had renal calculi composed of apatite associated with a disorder of calcium and phosphate metabolism. In the third case with apatite calculi the stones formed only in the bladder. Particular attention was paid to calcium and phosphorus metabolism in this dog which survived four years without any evidence of bone demineralisation. At the initial examination the serum calcium level was 9.5 mg/100 ml and serum inorganic phosphorus 3.1 mg/100 ml. A recurrent calculus was removed 28 months after the initial episode and on this

occasion it was composed mainly of calcium oxalate. At this latter time the serum calcium was 13.8 mg/100 ml, which is slightly above the normal limit found by Goulden (1969), and the serum inorganic phosphorus was 4.0 mg/100 ml.

In two other dogs with recurrent calcium calculi there was an interchange between calcium hydrogen phosphate and calcium oxalate, and this evidence tends to support McIntosh's (1942) suggestion that these calculi may have etiological factors in common.

d. Sex distribution of dogs with urolithiasis

The finding that calculi were more common in male dogs than in females is in close agreement with the series reported by Weaver (1970), and, although in his series the phosphate stones are not subdivided, there appears to be agreement that magnesium ammonium phosphate calculi are more common in the female while the other types of calculi are found mainly in male dogs.

e. Age distribution of dogs with urolithiasis

The mean age of dogs affected with each type of stone was found to agree with Weaver's (1970) series, although he stated that his figures did not show a statistically significant difference in the mean ages of the different stone types. In this study the differences were significant confirming the observation that calcium urolithiasis generally occurs in older animals while cystine calculi tend to occur in young animals and magnesium ammonium phosphate stones occur over a wide age range. The age distribution of magnesium ammonium phosphate calculi might be explained by the theory that this type of calculus is a consequence of urinary tract infection which can affect any age of dog.

In this study the age used was that when treatment in the Department of Veterinary Surgery was begun, but some animals had been treated previously for confirmed or suspected urolithiasis and others had developed clinical signs of

urinary calculi some considerable time before they were presented for treatment. Thus the age at which calculi first developed in dogs will be less than was found in this study but there is no evidence that the age relationship between the types of calculi would be substantially different.

f. Incidence of urinary infection with different types of calculi in dogs

The present finding of the frequent association of magnesium ammonium phosphate calculi with infection and the lack of association of other types of calculi with infection is in agreement with earlier studies in man and dogs (Krabbe, 1949; Bloom, 1954; Hellström, 1956; Carroll and Brennan, 1952a; Elliot, 1968; Hodgkinson, Peacock and Nicholson, 1969).

Some of the dogs received antibiotics before they were sent for surgical treatment, and this therapy probably altered the bacteriological findings. It was not possible to group these animals separately as inadequate information was available on the type of drug used or the time and duration of treatment but the numbers involved were small. The main effect of antibacterial therapy would be to increase the number of cases where no organisms were isolated or to reduce the number of bacterial species detected in mixed infections. There was no evidence that antibacterial agents were used more frequently in cases of any particular type of calculus and this factor seems unlikely to account for the different rates of infection with the different stone types. Slightly higher rates of infection for all types of calculi might have been found if antibacterial agents had not been used.

The more frequent occurrence of magnesium ammonium phosphate calculi in female than in male dogs may be due to a higher incidence of urinary infection in female animals. On the other hand it may be suggested that these calculi arise, from some other cause, in urine which is originally sterile and that

infection becomes established later. If the latter is correct infection might be more frequently found in female dogs with magnesium ammonium phosphate calculi than in male animals with this type of stone. In this study the incidence of infection was the same in both sexes and this evidence supports the theory that infection precedes magnesium ammonium phosphate stone formation.

g. Bacteria associated with different types of calculi in dogs

In this study a high incidence of staphylococcal infection was found in magnesium ammonium phosphate urolithiasis cases. This finding is in agreement with the results of other workers who have studied canine calculi (Milks, 1935; Brodey, 1955; Piermattei, 1960; Fritsch and Zuylen, 1966; Goulden, 1966).

Proteus species were isolated on 3 occasions, all associated with magnesium ammoniun phosphate calculi, but on only 1 occasion were these organisms present in pure culture. A similar low incidence of Proteus infection was observed by Brodey (1955) and Goulden (1966) and regardless of whether or not Proteus can contribute to magnesium ammonium phosphate calculi in dogs they are obviously not nearly so important as staphylococci.

Streptococci, E. coli and coliform organisms were isolated from a minority of cases of urolithiasis but did not appear to be of special significance.

No staphylococci were found in the urine of 13 cases with magnesium ammonium phosphate calculi. The absence of staphylococci may be due to their disappearance as a result of antibiotic therapy or it may be that in some dogs this type of calculus can form without the presence of these organisms.

h. Bacteria in the matrices of calculi from dogs

In reviewing the literature no report was found of histological examination of canine calculous matrix for the presence of bacteria although these techniques

had been used on calculi from mink (Nielsen, 1956).

Examination of stained sections showed bodies morphologically similar to cocci in 41 of the 43 magnesium ammonium phosphate calculi. Staphylococci or streptococci were isolated from urine in only 33 of these cases. This finding agrees with the results of Hyrntschak (1934) and Hellström (1936) who observed a higher incidence of staphylococci in human calculi than in urine examined at the time of treatment.

Histological examination cannot confirm that these Gram-stained bodies are bacteria but they closely resemble bacteria and it seems likely that they are either staphylococci or streptococci. Their presence in such a high proportion of magnesium ammonium phosphate stones indicates that they are of significance in the etiology of this type of calculus.

The absence of bacteria from the matrices of two calculi from cases 38 and 38R suggests that in some circumstances magnesium ammonium phosphate calculi can form without the presence of staphylococci and this observation is in agreement with the results of Nielsen's (1956) studies on mink where a minority of calculi formed without evidence of infection.

It was also interesting to note that no bacteria other than cocci were seen in the stones and that no organisms of any type were seen in the matrices of calcium oxalate or cystine stones. If bacteria are merely an accidental inclusion in calculi they ought to have been seen in the matrices and the exclusive presence of cocci further supports the view that they are of etiological importance in magnesium ammonium phosphate stones.

i. Recurrence of urolithiasis in dogs

Study of the earlier literature on urolithiasis failed to reveal any reports on the recurrence of canine stones where a sufficiently large number of

dogs had been examined over a reasonably long period after the initial episode. The studies of Krabbe (1949) and Weaver (1970) seemed to be deficient in respect of the criteria of recurrence as these authors measured recurrence only as the return of clinical signs. Although clinical recurrence is important as far as the animal and its owner are concerned, it seems that radiological evidence of recurrent urolithiasis would be a more accurate and reliable measure. The presence of clinical signs is variable depending on large calculi causing bladder irritation or on small stones obstructing the urethra. In most of the dogs studied, radiological evidence of urinary calculi was found before clinical signs developed and in some animals calculi were present in the bladder for months or years without producing any obvious change in the animal's behaviour. A similar observation was made by Stainton (1922).

In calculating the recurrence rate for urolithiasis it is assumed that urinary calculi did not spontaneously disappear from the urinary tract subsequent to being detected radiographically. No evidence of such spontaneous disappearance was noted in dogs examined on several occasions; indeed it was noticed that the calculi generally increased in size.

If magnesium ammonium phosphate calculi are caused by infection and calcium and cystine stones are not the result of infection, it might be expected that antibiotic treatment would reduce the recurrence of magnesium ammonium phosphate but have no effect on the other types of calculi. In the present study, dogs with magnesium ammonium phosphate calculi were less likely to develop recurrent urolithiasis than animals with calcium or cystine stones. Although Weaver (1970) failed to find a statistically significant difference in the recurrence of different types of stones his results agree with the present study in finding fewer recurrences in the phosphate group, which, in his series, may have included

magnesium ammonium phosphate, calcium hydrogen phosphate and apatite.

There are three possible explanations for the 30% recurrence of magnesium ammonium phosphate calculi. Firstly, the antibiotic therapy may have failed to clear the infection and in several cases there was evidence that staphylococci persisted in the urinary tract or that they quickly became re-established. Secondly, there may be factors other than bacteria which cause this type of calculus. One dog is recorded in this investigation where the initial and recurrent calculi were not associated with urinary infection and bacteria were not found in the matrices of the calculi. Thirdly, the recurrent calculi are sometimes of different composition from the initial stone and they may therefore be due to different predisposing factors.

The recurrence rate of renal calcium stones in man reported by Williams (1969) is very similar to the results obtained for calcium calculi in the present study on dogs. The high rate of recurrence of calcium calculi and cystine calculi was not unexpected as no treatment had been given which might have affected the predisposing causes.

j. Bacteria associated with recurrent calculi in dogs

Dogs which had magnesium ammonium phosphate calculi at the initial episode were more likely to have urinary infection at recurrence than the other groups. Staphylococci were isolated from 9 out of 10 cases where recurrence was associated with infection and these results conform with the findings of Brodey (1955). The results also agree with the observation that recurrent phosphate calculi in man are associated with staphylococcal infection. (Hellström, 1936; Higgins, 1939).

Coliform organisms were isolated from 4 dogs at follow-up examination but none of these animals had recurrent calculi. This finding agrees with the

observation in human patients that recurrence did not take place with this type of infection (Hellström, 1936).

In this investigation more conclusive evidence regarding the significance of staphylococci in recurrent urolithiasis might have been obtained had it been practicable to divide the magnesium ammonium phosphate cases into two groups and treat only one group with antibiotics. However on ethical grounds antibiotic treatment was given when urinary infection was present.

k. Composition of recurrent calculi in dogs

Analysis of 19 recurrent calculi showed that 14 of them were mainly of the same composition as the initial stones. No previous studies on recurrent canine calculi were found with which to compare these results, but findings similar to the present study have been reported in human urolithiasis (Keyser, 1934; Hodgkinson, Peacock and Nicholson, 1969).

Calculi differing in composition from the initial stones were recorded in 5 cases involving 4 dogs. In 4 cases recurrence can be explained by staphylococcal infection becoming established after removal of calcium or cystine stones. In case 42A, after antibiotic treatment, cystine calculi recurred.

The results of observations on 2 cases with cystine calculi may indicate that high urinary pH had an effect in preventing the formation of cystine stones but the fact that magnesium ammonium phosphate stones did not develop suggests that factors other than alkaline urine are necessary for the development of this latter type of calculus.

l. Treatment of recurrent magnesium ammonium phosphate calculi in dogs

If recurrence of magnesium ammonium phosphate calculi is partly due to inadequate antibiotic therapy it should be possible to reduce further stone

formation if the infection is cleared. Only two cases were available for prolonged therapy and in both there was no recurrence after the staphylococci had been eliminated from the urine.

m. Rate of development of recurrent calculi in dogs

The present investigation was not designed to study the rate of stone growth. Such information could only be obtained by carrying out radiological examinations at regular intervals and much more frequently than was done in this investigation. However while carrying out the follow-up studies an impression was formed that magnesium ammonium phosphate and cystine calculi re-grow more quickly than calcium stones. The results of the times at which recurrent calculi were detected are inadequate for analysis but seem to indicate that calcium calculi do reform at a slower rate than the other types.

The relatively slow rate of growth of recurrent calcium calculi and the fact that they occur mainly in older dogs is of some importance as many of these animals die of other diseases before the recurrent calculi produce clinical signs.

n. Leucocyte counts in dogs

There was considerable variation in counts obtained from the dogs in this study but the mean count from dogs with urinary infection was higher than the mean count from those without infection, and a greater difference was detected when the count from cases with urinary obstruction was compared with the count from those free of obstruction. Urinary obstruction therefore produced a more marked leucocyte response than urinary infection.

Dogs with neither urinary infection nor urinary obstruction had a mean leucocyte count within the normal ranges found by Doxey (1964) and Schalm (1965) while the mean count from dogs with both infection and obstruction was greater than the mean plus two standard deviations as recorded by these authors.

o. Infection and urolithiasis in cats

Examination of the crystalline material from 10 cats showed that in every case it was mainly composed of magnesium ammonium phosphate. In 9 of the cases no bacteria were isolated from the urine, a result in agreement with the findings of Rich and Kirk (1969) and Schechter (1970). Notwithstanding the reports of Fishler (1955), Foster (1967) and Meier (1967) it would appear that bacteria are not a cause of urolithiasis in cats.

p. Infection and urolithiasis in sheep

X-ray diffraction analysis of the crystalline material from 7 sheep showed that in 4 cases the material was mainly magnesium ammonium phosphate while in the remaining 3 cases it could not be identified. Similar difficulties in the analysis of sheep calculi were encountered by Sutor and Wooley (1970). Silica has been detected by chemical analysis in urinary calculi obtained from sheep at autopsy by the Department of Veterinary Pathology, Royal (Dick) School of Veterinary Studies and reported in sheep by Trueman and Stacy (1969).

There appears to be at least two different types of calculi in sheep and these may differ in etiology. In this investigation the numbers available are too small to draw firm conclusions but there does not seem to be any relationship between infection and urolithiasis in sheep.

5. CONCLUSIONS

In this study, as in surveys by other workers, magnesium ammonium phosphate was the most commonly identified constituent of canine calculi. A higher incidence of calcium oxalate calculi was recorded in the present series than in other surveys. The age and sex distribution of dogs with urolithiasis and the position of calculi in the urinary tract conformed with other studies on dogs in Britain.

Magnesium ammonium phosphate calculi were associated with urinary infection in 82% of cases while infection was detected in only 22% of calcium stone cases and was absent in all cystine urolithiasis cases.

In 44 cases of magnesium ammonium phosphate calculi staphylococci were isolated on 31 occasions, streptococci on 7, Proteus on 3 and other organisms on 5 occasions while in 8 cases the urine was sterile.

In 41 out of 43 magnesium ammonium phosphate calculi, bodies which resembled bacterial cocci were found in the matrices. Cocci were more frequently found in the calculi than isolated from urine. On 2 occasions bacteria were not detected in the urine or in the calculi. These findings indicate that although staphylococci are very frequently associated with magnesium ammonium phosphate calculi they may not always be present.

Recurrence of magnesium ammonium phosphate calculi was less common than calcium or cystine stones and this result may be attributed to the use of antibiotics to treat urinary infection.

At follow-up examination 11 dogs were infected with staphylococci and 9 of these had recurrent calculi composed of magnesium ammonium phosphate.

The composition of the recurrent calculi was usually similar to that of the original stones except in cases where staphylococcal infection had become

established. These dogs all developed magnesium ammonium phosphate calculi.

Examination of urine samples and crystalline material from 10 cats and 4 sheep failed to find any association between infection and magnesium ammonium phosphate crystal formation.

PART II Physical characteristics of canine urinary calculi

The weights, numbers, shapes and surface appearances of magnesium ammonium phosphate calculi removed from dogs were compared with observations made by other workers. The factors which might influence the physical characteristics of urinary calculi are considered and discussed.

The internal structure of magnesium ammonium phosphate stones was also studied and the results compared with the observations on canine and human calculi published by other workers.

1. REVIEW OF THE LITERATURE

a. Weight of urinary calculi

Examination of the literature failed to reveal any reports on the weights of calculi formed in the canine urinary tract, although Blount (1931) recorded one case where a calculus weighing 490g had been removed.

Hodgkinson, Peacock and Nicholson (1969) studied the weights of calculi formed in the human urinary tract and recorded the distribution of the weights of 413 calculi from male patients and 151 calculi from female patients. This record showed that 72% of stones from male patients weighed less than 0.3g and 55% less than 0.1g. The corresponding figures for female patients were 61% and 36% indicating that there was a higher proportion of larger stones in women than in men. These authors did not state the number of patients involved so it is possible that some patients produced more than one calculus.

The composition of the calculi in relation to their weight was also studied by Hodgkinson et al. who found that the small stones consisted mainly of calcium oxalate while in the heavier calculi magnesium ammonium phosphate and calcium phosphate predominated. Thompson, Steadman, Benjamin and Scott (1944) also noted that larger human calculi contained more phosphate while smaller calculi contained more oxalate.

The relative density of different types of urinary calculi does not appear to have been studied although Milks (1935) stated that calculi composed of magnesium ammonium phosphate were relatively lighter in weight than the other types but he did not publish evidence to support his observation.

b. Number of calculi

Krabbe (1949) described 30 cases of canine urolithiasis and noted that 21 of the dogs had only one calculus, 4 dogs had two calculi and 5 dogs had more

than two including a dog with over 500 stones. Bloom (1954) observed that phosphate calculi could be present as large single calculi, large multiple calculi or numerous small calculi reaching into hundreds. White, Treacher and Porter (1961) also observed that phosphate calculi could vary in number from a single calculus to several hundred.

c. Shape and surface texture of calculi

McCunn (1947) published a photograph of a single bladder calculus from a dog showing it to be ovoid in shape while two other stones, from a dog with several vesical calculi, had flattened surfaces. He considered that these facets were produced by the surfaces of adjacent stones rubbing together. White, Treacher and Porter (1961) noted that single phosphate calculi usually had a rough surface while multiple calculi were usually smooth, faceted and tetrahedral. White (1966) noted that a single stone in the renal pelvis or bladder may take the form of the cavity within which it develops. He also observed that the surfaces of calculi may be smooth and polished or rough and coral-like.

Three types of phosphate calculi were described by Goulden (1966). The first type were smooth, numerous, irregularly spherical with a smooth or slightly roughened surface. The second type occurred as large single calculi, spherical but with some degree of flattening and with a rough surface, while the third type, which usually occurred as a few large vesical calculi, were tetrahedral with smooth surfaces and rounded corners.

The variation in the shape of human bladder calculi was noted by Lonsdale (1968a) who observed that some were ellipsoidal, others were asymetrical or roughly spherical and a third type were rounded tetrahedra, especially when a number of stones were packed tightly together.

Haugh, Lonsdale, Mason and White (1966) considered that in cases of canine

urolithiasis where multiple stones were present they tended to pack together in the bladder and if deposition of crystals continued spherical calculi would tend to become tetrahedra by continued growth rather than by friction. They pointed out that tetrahedra pack more efficiently into a given space than do spheres. Haugh et al. also mentioned the possibility that the tetrahedral growth of a calculus could be produced by orientation of the crystals as they formed on the surface of the stone.

Several authors commented on the surface texture and colour of canine phosphate calculi. Blount (1931) and Lauder (1949) observed that they had a smooth surface while Bloom (1954) noted that the surface could be smooth or rough. There appears to be general agreement that these calculi are coloured shades of white or grey. (Blount, 1931; Milks, 1935; Bloom, 1954; White, Treacher and Porter, 1961; Smith and Jones, 1966; Goulden, 1966).

d. Internal structure of calculi

The simplest method of examining the internal structure of calculi is to break or cut them, macroscopic examination of the exposed material often revealing a symmetrical arrangement of concentric rings. Study of the central part of the stone may show that it differs in composition from the rest of the calculus.

Thin cross-sections of canine calculi were examined by Cornelius and Bishop (1961) who photographed the unstained sections to show their internal structure. These authors also carried out histochemical tests and staining methods on the sections to identify the nature and distribution of some of the organic material in the stone matrices. Similar procedures were carried out on calculi of human origin by Boyce, Pool, Meschan and King (1958) and the similarity in structure between human and canine calculi was noted by Cornelius

and Bishop (1961).

Sections of calculi from human patients were studied by Ord and Shattock (1895) using polarised light and this technique was also used on stone fragments by Prien and Frondel (1947). The use of the polarised light microscope in the study of sections of human calculi was discussed by Murphy and Pyrah (1962), but this technique does not appear to have been used on canine stones although Trueman and Stacy (1969) used it in the study of ovine uroliths.

X-rays have been used to study calculi and variations in radio-density in different parts of the stones may be detected and so yield information about the structure of the calculi. Trueman and Stacy (1969) carried out radiological examination of intact calculi from sheep but more useful information has been obtained by cutting thin sections of the stones, preparing radiographs of them and then enlarging the films. This technique is known as microradiography and its application in biology has been discussed by Cosslett and Nixon (1960). Sections of human urinary calculi have been subjected to this examination (Lagergren, 1956; Boyce, Pool, Meschan and King, 1958; Murphy and Pyrah, 1962).

Hedenberg, Engfeldt and Engström (1953) used microradiography to study calculi produced experimentally in rats but there is no record of canine stones being examined in this manner.

Identification of the crystalline constituents of calculi by their relative radio-densities was mentioned by Lagergren (1956) using techniques developed by Engström (1946). Lagergren confirmed the results of microradiography by analysing small portions of the stone sections by X-ray diffraction crystallography. He found that although human calculi often contained more than one crystalline component, only a few combinations occurred. In calculi which contained more than one crystalline constituent, each substance was usually deposited in distinct

areas within the stone and complete intermixing of crystals did not occur.

Murphy and Pyrah (1962) studied the distribution of the monohydrate and dihydrate salts in calcium oxalate calculi from human patients. They found that the monohydrate salt was situated towards the interior of the stone while the outside layer was always composed of dihydrate crystals. Murphy and Pyrah postulated that only dihydrate crystals were deposited from urine and that re-crystallization within the calculus produced the monohydrate form.

The main structural features which have been recognised in calculi are nuclei, concentric lamination, radial striation, column formation and fissures.

i. Nuclei

It is generally agreed that a urinary calculus enlarges by the progressive deposition of crystals on its surface and the point of origin of the calculus is the nucleus. In some calculi the central part differs in appearance and composition from the rest of the calculus. There is, however, considerable controversy regarding the nature and significance of nuclei.

Morton (1844) stated that calculi from domestic animals contained nuclei which rarely differed in composition from the rest of the stone and observed that nuclei composed of foreign material were uncommon. According to Stainton (1922) the theory that a calculus was formed by deposition round a nucleus was first proposed by Antoine de Neyde in 1686 but Stainton did not give the source of this information. The role of the nucleus in urolithiasis in the domestic animals was discussed by Blount (1931) who considered that once a calculus has started to form on a nucleus growth can continue although there is no abnormality of urine composition. Milks (1935) noted that infection may aid the formation of phosphate calculi by providing nuclei and this view was supported by Smith and Jones (1966). Trueman and Stacy (1969) found nuclei in several sheep calculi

which were composed of layers of calcium oxalate, calcium carbonate and silica.

Prien (1949) did not think that desquamated epithelial cells were important as nuclei in human calculi although in a further study (Prien, 1955) he found that nuclei were usually present in calculi from human patients. These nuclei were frequently composed of calcium phosphate and Prien considered that the nature of the nucleus did not govern the kind of substance precipitated on it. Lagergren (1956) found that human calculi might or might not have nuclei. He suggested that in the stones without a visible nucleus a single crystal or group of crystals may have acted as a centre for precipitation. In the calculi which had nuclei he often found that the nuclei absorbed less X-rays than the rest of the stones and suggested that the nuclei consisted of organic material such as blood clot, epithelial cells or bacteria.

The organic matrices of human calculi were examined by Boyce and Sulkin (1956) who failed to find any nidus of cellular debris, bacteria or foreign material. However many calculi contained multiple nuclei consisting of compact whorls of organic matrix. Maurice and Henneman (1961) also considered that it was uncommon for the nuclei of stones to be composed of bacteria or epithelial cells. Murphy and Pyrah (1962) defined the stone nucleus as "a focal point or area which differs in composition from the rest of the stone", and noted that calculi which had such nuclei were in the minority in their series of cases. The nuclei which they observed consisted of necrotic tissue, organic colloidal material or an aggregate of crystals, and they found that small calculi of urate and oxalate may serve as the centres of growth for oxalate and magnesium ammonium phosphate calculi respectively.

Lonsdale (1968a) observed that some human bladder calculi had well defined nuclei with successive layers of crystalline material radiating from the nuclei

while others were conglomerates of crystals with no obvious nuclei. Aggregation of calcium oxalate crystals was considered by Hodgkinson, Peacock and Nicholson (1969) to be the initial step in the formation of many human calculi and they felt that this observation was supported by their finding that very small calculi consisted almost entirely of calcium oxalate.

ii. Lamination

Bloom (1954) noticed that many canine calculi were composed of concentric layers and he considered that the layers varied in composition according to the reaction of the urine at the time the salts were deposited. Concentric laminae in canine phosphate calculi were noted by Goulden (1966) and by Cornelius and Bishop (1961). The latter authors found that crystals within these layers were present either as typical magnesium ammonium phosphate crystals or as amorphous material, and from studies on decrystallized calculi they found that the organic matrices were arranged as concentric laminae of dense fibrillar material with an amorphous material between the layers.

Some of the ovine calculi studied by Trueman and Stacy (1969) were made up of layers of differing mineral composition. These workers observed changes in calcium excretion, urine flow and urinary pH after feeding and they considered that these changes could be responsible for the deposition of minerals of differing chemical stabilities.

In thin sections of human urinary calculi Lagergren (1956) found, with very few exceptions, that two or more components in the same calculus occurred as discretely separated layers. By using X-ray micro-diffraction analysis he confirmed that individual laminae consisted of a single crystalline component. Lagergren thought that the arrangement of the laminae reflected changes in the ionic milieu of the urine during the formation of the calculus. Lonsdale (1968a)

noted the presence of layers resembling "tree-rings" in many human calculi and suggested that they corresponded with diurnal or seasonal variations in pH. In another paper (Lonsdale, 1968b) she discussed the possible growth of one crystal on a substrate of another with a near geometrical fit between respective networks which are in contact. This type of growth is known as epitaxy and when Lonsdale (1968b) examined the crystal habits of the common stone-forming components and the dimensions of the networks she found that epitaxial growth could occur on a considerable number of the crystals found in calculi.

Boyce and King (1959) studied the distribution of the organic matrix material in human calculi and the relationship of crystals to this material. They found that laminae of dense matrix surrounded the centre of the calculus at more or less regularly spaced intervals. By examining serial sections these workers confirmed that each lamina formed a complete membrane round the stone. The spaces between laminae were filled by amorphous matrix material. Boyce and King (1959) observed that crystals of apatite were deposited along the laminar matrix while magnesium ammonium phosphate crystals were present with amorphous matrix between the laminar bands. This arrangement was also noted by Murphy and Pyrah (1962) who found that calculi containing magnesium ammonium phosphate usually had a laminar structure although this was not always apparent to the unaided eye. Studies on the solubility of apatite in urine by Prien (1949) showed that this material could be precipitated over a wide range of urinary pH.

Concentric laminae were seen in human calculi composed of calcium oxalate monohydrate by Lagergren (1956) and by Murphy and Pyrah (1962). Boyce, Pool, Meschan and King (1958) found laminae on macroscopic examination of human cystine calculi but these bands were not seen on microradiographic examination.

iii. Radial striation

Goulden (1966) observed striations radiating from the centre of tetrahedral canine calculi composed of magnesium ammonium phosphate and this arrangement is also seen in a photograph of a similar calculus published by Cornelius and Bishop (1961).

Carr (1953) found radial striations in 42% of 500 calculi from human patients. These striations were found only in areas which consisted of calcium oxalate monohydrate, calcium hydrogen phosphate dihydrate and uric acid. They were always associated with concentric laminae. These findings were supported by Lagergren (1956) who added that calculi composed of tricalcium phosphate and ammonium hydrogen urate could also show radial striations. Murphy and Pyrah (1962) did not find radial striation in magnesium ammonium phosphate calculi although the fractured surface of one stone appeared so arranged, but on examination of this section the apparent striation was due to physical weakness parallel to the line of development of single crystals.

Boyce (1969) found that each radial striation in human calculi contained a layer of matrix which originated at right angles to the matrix of a concentric lamina and, after crossing several laminae, ended in the matrix of a further lamina. He considered that this formation could be explained by cross linking between molecules in the laminar matrix.

iv. Column formation

Lagergren (1956) observed that in some human calculi magnesium ammonium phosphate crystals were distributed in a loose trabecular network, and in fresh specimens fluid filled some of the intertrabecular cavities. He noticed well formed magnesium ammonium phosphate crystals up to several millimetres in size in some calculi. A similar arrangement was noted in a small number of magnesium

ammonium phosphate calculi by Murphy and Pyrah (1962) who described columns of these crystals growing in an outward direction. These stones were laminated and laminae of colloid matrix and apatite cut through the columns. Colloid matrix and apatite also formed a coating round each column.

v. Fissures

Prien (1955) noted that cavities partly lined with calcium phosphate were frequently present in the interior of human calculi but he was unable to understand how calcium phosphate could be dissolved and removed through the dense outer layers of the calculi. Lagergren (1956) observed that a human calculus composed of magnesium ammonium phosphate contained a cavity whose walls were covered by dried blood and Boyce, Pool, Meschan and King (1958) published, without comment, a photograph of a human apatite stone showing a large central fissure.

Spontaneous disintegration of human calculi within the bladder was recorded by Prout (1848) and Keyser (1923) but no reference was found to this occurring in animals.

2. MATERIALS AND METHODS

a. Collection of calculi

The calculi removed from the urinary tract were thoroughly washed. The shape and number of the stones were recorded. They were allowed to dry at room temperature for at least 8 weeks before measuring the total weight of all calculi removed from each dog. In cases of magnesium ammonium phosphate calculi the weight of the largest stone was also obtained.

b. Density of calculi

Eight fairly large calculi were selected and their specific gravity determined in the following manner. The stones were immersed in a small volume of water for several weeks, the surfaces were then dried with blotting paper and the calculi weighed; their volume was then measured by the volume of water they displaced.

c. Radiographic examination of calculi

Representative samples of calculi from 43 cases of magnesium ammonium phosphate urolithiasis were arranged in groups of approximately similar size and radiographs of them obtained on Kodak "Kodirex" film, using a focal film distance of 76 cm with exposures of 60 kV, 100 mA and 0.08 - 0.20 seconds. The calculi were turned through an angle of 90° to the X-ray beam and a second film exposed. The films were developed using standard X-ray film developer and fixer.

d. Preparation of thin sections of calculi

Calculi representative of the different shapes of magnesium ammonium phosphate stones were selected. Calcium oxalate, apatite, cystine and ammonium urate calculi were taken for comparison and contrast. Stones larger than 1.5 cm were

cut into halves or quarters using a rotating diamond disk cooled by water. Initial attempts to cut thin sections from canine calculi showed that they generally tended to crumble so it was decided to impregnate them in order to prevent this disintegration.

Clark and Iball (1957) used "Marco" resin for embedding bone prior to microradiography and Boyce et al. (1958) used "Carolina embedding plastic" for human calculi. In the present study calculi were embedded in methyl methacrylate using slight modifications to the techniques described by Smale (1956) and Murphy and Pyrah (1962).

Liquid methyl methacrylate was washed three times with a 5% solution of sodium hydroxide to remove hydroquinone which had been added to inhibit polymerisation. The methyl methacrylate was washed four times with distilled water to remove all traces of sodium hydroxide, then 0.5g benzoyl peroxide was added to 100 ml to catalyse the polymerisation reaction. Calcium chloride was also added to remove water and the liquid methyl methacrylate stored at $4^{o}C$.

The stone specimens were covered with methyl methacrylate in suitable glass tubes connected to a vacuum pump which reduced the pressure in the tubes to 0.1 mm mercury. This low pressure was maintained until no more air bubbles were released from the stones. The methyl methacrylate was then polymerised in a water bath at $40^{o}C$ for several hours. The resulting embedded specimens were mounted on a jig which moved very slowly towards a rapidly spinning diamond impregnated disk cooled by water jets. Using this apparatus sections were obtained which ranged in thickness from 70 to 350 μm. The sections were not ground or polished as they were thin enough for examination.

e. Microradiographic examination of sections of calculi

The sections were mounted on adhesive plastic tape ("Sellotape") which was then fixed to strips of X-ray film from which the emulsion had been removed. The sections were placed in front of a Kodak special high resolution film for microradiography, the specimens being separated from the film emulsion only by the layer of tape 50 μm thick and a layer of paper 120 μm thick. Exposures were made using a horizontal X-ray beam from a Machlett OEG - 50 X-ray tube with a focal spot 1.5 mm diameter and inherent filtration of 1.0 mm beryllium. The focal-film distance was 60 cm and the exposure factors were 20 kV, 30 mA and 750 seconds. The films were developed in universal photographic developer and photographic enlargements made.

Lagergren (1956) used sections of similar thickness but his X-ray machine had a different target diameter and he used a shorter focal-film distance. He calculated that using the most adverse factors the geometric blurring on the film did not exceed 3 μm, while Murphy and Pyrah (1962) using sections of about 100 μm obtained sufficient resolving power to allow a useful magnification of about 50 times. Using the thickest section in this study and allowing 170 μm between the section and the film it was calculated that the geometric blurring would be less than 2 μm.

Methyl methacrylate did show slightly on the X-ray films but there was sufficient contrast in radio-density between the crystals in the calculi and the methacrylate.

f. Macroscopic examination of sections of calculi

The sections prepared for microradiography were examined at low magnification using a hand lens. Some of the sections were photographed and the films enlarged.

3. RESULTS

a. Weight of urinary calculi

Weights were obtained for the total amount of stones removed from 47 cases of magnesium ammonium phosphate calculi, 38 cases of calcium and 22 cases of cystine including in these totals 12 cases of recurrent calculi. The weights of the calculi removed from individual dogs are given in Appendix 5. The distribution of the weights of these three types of calculi is given in Fig. 15 and the results are summarised in Table 25 which gives the mean weight of each type of calculus and, where sufficient results were available, the mean weight of each stone type from male and female dogs.

Table 25

Mean weights in grams of different types of calculi removed from clinical cases

Type of calculi	Sex	No. of cases	Mean weight
Magnesium ammonium phosphate	Both	47	15.45
Magnesium ammonium phosphate	Female	36	19.87
Magnesium ammonium phosphate	Male	11	0.98
Calcium calculi	Both	38	0.50
Calcium calculi	Male	33	0.43
Cystine calculi	Male	22	0.50

Examination of the distribution of the weights of the calculi (Fig. 15)

showed that as the stone weight increased the number of cases became less. A greater weight of calculus was produced in dogs with magnesium ammonium phosphate urolithiasis than in those with calcium or cystine calculi. This difference was less when only calculi from male animals were compared. Magnesium ammonium phosphate calculi from female dogs were considerably heavier than those from male animals.

The weight of the largest single calculus removed at each episode from dogs with magnesium ammonium phosphate stones is recorded in Appendix 6. The largest calculus in this study weighed 140g and was removed from the dog with the greatest weight of calculi, 371g.

b. Density of calculi

The specific gravities of 4 magnesium ammonium phosphate, 2 calcium oxalate and 2 cystine calculi are recorded in Table 26.

Table 26

Specific gravity of canine calculi

Magnesium ammonium phosphate	Calcium oxalate	Cystine
1.22	1.61	1.55
1.25	2.20	1.55
1.48		
1.54		

c. Number, shape and surface texture of magnesium ammonium phosphate calculi

The shape and surface appearance of magnesium ammonium phosphate calculi appeared to be partly related to the number of calculi present. In this study

magnesium ammonium phosphate stones from 49 episodes including 5 cases of recurrent urolithiasis were studied. Illustrations of the different appearances of these calculi are given in Figs. 16 - 25.

In 10 cases only single calculi were present and they were spheroidal being flattened on the dorsal and ventral aspects. In a further 7 cases a single large calculus was accompanied by several much smaller calculi. When several large calculi were present they were usually tetrahedral and a radiograph illustrating the arrangement of these calculi in the bladder is given in Fig. 26. Multiple small tetrahedral calculi were also observed in some cases and in others the small calculi were spherical with small facets. It was noted however that the ammonium urate calculi from case 103 were almost perfect spheres although fairly large multiple calculi were present.

Evidence that magnesium ammonium phosphate calculi tend to adopt the shape of the organ in which they form is given in Fig. 27 which shows that a large calculus had adopted the outline of the renal pelvis in which it developed. This calculus was removed from a dog presented before this study was begun.

Large single calculi had rough surfaces probably corresponding to the folds of the hypertrophied mucous membrane of the bladder. When several large stones were present or when a single large calculus was accompanied by several small calculi the surfaces of the stones in contact with each other were smooth (Figs. 16 - 20).

Magnesium ammonium phosphate calculi were mainly creamy white in colour although some were whiter than others.

d. Radiographic examination of calculi

Radiographic examination was carried out on calculi from 43 cases of magnesium ammonium phosphate urolithiasis including 4 recurrent cases. The

results are presented in Figs. 28 - 30 but some of the detailed structure seen on the original radiographs has been lost in the photographic illustrations.

The films were examined for variations in radio-density throughout the calculi. In case 41A (Fig. 29) the centres of the stones were less dense than the surrounding layers while in case 19 (Fig. 30) the centre was more radio-opaque. However, in the majority of calculi the radio-density of the centres of the stones resembled that of the surrounding layers and distinct nuclei could not be detected by the use of X-rays.

Rings of material with differing radio-density were seen in cases 10, 16, 30, 40 and 41A and these probably correspond with laminae in these calculi.

Several calculi showed cracks or fissures where no radio-dense material was present; this arrangement was noted only in tetrahedral or irregularly tetrahedral calculi with the exception of case 24 where an ovoid stone had a large fissure. The fissure in this case was observed in the calculus prior to removal from the bladder (Fig. 31) and the cut surfaces revealing the fissure are shown in Fig. 32.

The large spherical calculi from cases 31, 32 and 37 showed an even distribution of radio-dense crystals with a considerable amount of radio-lucent material between them. The calculus with sharp surface projections from case 38 appeared to consist of an aggregate of sharp spicules.

e. Microradiographic and macroscopic examinations of sections of calculi

Thin sections were prepared from 18 calculi. The chemical composition of these stones is given in Table 27.

Table 27

The chemical composition of calculi examined by thin section techniques

Type of calculus	Number examined	Case numbers
Magnesium ammonium phosphate	11	5, 7, 10, 15, 30, 33, 34, 38, 40, 41A, 42A
Calcium oxalate	3	44, 68, 78
Apatite	1	79
Cystine	2	41, 95
Urate	1	103

The microradiographic and macroscopic appearance of examples of these calculi are illustrated in Figs. 33 - 40a.

i. Magnesium ammonium phosphate

Macroscopic examination of sections of 11 magnesium ammonium phosphate calculi failed to reveal nuclei in 9 of the stones but in 2 cases (41A, 42A) the central parts of the calculi were of a different colour and consistency from the surrounding structures. Microradiographic studies failed to show any difference in radio-density between the central and peripheral parts of the calculi but in case 42A (Fig. 33) the central part of the stone was well defined. In cases 41A and 42A magnesium ammonium phosphate calculi formed in dogs which had previously developed cystine calculi and it is probable that the central parts of these stones were composed of cystine.

Concentric laminae were found in 10 of the sections on macroscopic examination and were revealed by microradiography on 8 occasions. (Figs. 34, 35) The number and width of the laminar bands varied considerably and most of the calculi had

areas without laminae. On the microradiographs, bands of radio-dense material, probably composed of apatite, alternated with less radio-opaque bands of magnesium ammonium phosphate and in most cases these bands became more frequent and closer together towards the periphery of the calculi.

In the section from case 5 (Fig. 34) the laminae in the centre of the section were circular while those towards the periphery were triangular suggesting that the calculus had originally been spherical and had later developed its tetrahedral shape.

On macroscopic examination, striations radiating in a regular manner from the centres of the calculi were not seen although in two (cases 10, 15) some radiating lines of a darker colour were present. A radial distribution of crystals was not seen on microradiography although in cases 7 and 10 there were numerous cracks in a radial arrangement.

Columnar or trabecular distribution of magnesium ammonium phosphate crystals was seen to some extent in case 10 although areas where magnesium ammonium phosphate crystals appeared as loose aggregates were seen in 6 other calculi. Comparison of these sections with decrystallized sections of matrix prepared in Part I of this study showed that the matrix material extended round and between the magnesium ammonium phosphate crystals.

Fissures or cracks were clearly seen on microradiographs of several of the sections but it was difficult to tell defects in the crystallization of the calculi from fractures produced during the preparation of sections. The fissures either radiated from the centre of the calculi or lay between and parallel to the concentric laminae.

The calculus from case 38 differed in several respects from the other magnesium ammonium phosphate calculi (Fig. 36) in that there was no evidence of

lamination but the stone was composed of elongated and pointed crystalline masses projecting upwards and outwards from a flat base. Small amounts of radio-dense material, probably apatite, were present but there was also non-crystalline material between the sharp projections.

ii. Calcium oxalate

In case 44 the centre of the stone appeared lighter in colour and more radio-dense than the surrounding layers while in the other two calculi examined no distinct centre could be seen. These calculi did not show concentric laminae as noted in magnesium ammonium phosphate stones although in the sections the central area was surrounded by a band of a different material (Fig. 37). In the central area of the calculus from case 44 the crystals appeared to have a radial orientation and the crystals projecting from the periphery of calcium oxalate calculi were also radially arranged. These calculi contained radio-dense material, probably apatite, which was not distributed in any recognisable pattern but occurred as deposits in several areas of the calculi. Fissures were not present but the central areas contained some non-radio-opaque material.

X-ray crystallographic analysis of the calculus from case 68 showed it to be composed of calcium oxalate dihydrate with a lesser amount of calcium oxalate monohydrate while the stone from case 78 was mainly composed of calcium oxalate monohydrate with a small amount of calcium oxalate dihydrate. The distribution of the two types of calcium oxalate is seen on microradiography (Fig. 37) where the more radio-dense monohydrate salt is in the central part of the calculus and the dihydrate salt distributed around the outside.

iii. Apatite

Only one calculus containing a large proportion of apatite was examined and the central area was found to be of a lighter colour and less radio-dense than

the peripheral part of the stone (Fig. 38). The central area contained radio-lucent material interspersed with some moderately dense crystals. There was a single peripheral layer and from it radio-dense, closely packed crystals projected in a radial arrangement.

iv. Cystine

Two cystine calculi were examined. One of them contained a central area which was lighter in colour than the surrounding layers but it was not distinguished on microradiographic examination (Fig. 39). Concentric laminae were clearly visible on macroscopical examination but not on microradiography where the sections appeared to be of almost uniform radio-density. There was no evidence of radial striations or fissures and no areas of radio-lucent material.

v. Ammonium urate

One ammonium urate calculus was examined and although the centre of the calculus was darker in colour it did not differ in radio-density from the surrounding parts of the stone (Fig. 40). Numerous laminae were present, some of the bands containing no radio-dense material. Radial striations were observed in one band but were not present in the rest of the calculus and no fissures were seen.

4. DISCUSSION

a. Weight of urinary calculi

In this study it was found that the total weight of calculi removed from the urinary tract at each episode was related both to the sex of the animal and to the chemical composition of the stones. The calculi which formed in female dogs were much heavier than those developing in male animals; this may be explained by the difference in the length and diameter of the urethra in the two sexes. The long narrow urethra of the male dog is relatively easily blocked by a smaller calculus than the shorter wider urethra of the female which allows larger stones to pass.

When the effect of sex differences was eliminated by comparing only calculi which formed in male animals, it was found that the magnesium ammonium phosphate calculi were heavier than the calcium or cystine stones. This difference is difficult to explain except that magnesium ammonium phosphate calculi may grow more quickly than the others and thus are larger or more numerous before clinical signs develop.

Hodgkinson, Peacock and Nicholson (1969) studied the weights of individual human calculi rather than the total weight of stones removed at each episode. Their results conformed with this study in that a higher proportion of large calculi occurred in females than in males and on analysis of the calculi they found that the smallest calculi consisted almost entirely of calcium oxalate while the larger stones were mainly composed of calcium phosphate or magnesium ammonium phosphate.

Owing to the difficulties in producing calculi in experimental animals it was not possible to study the rate of growth, i.e. the relationship between stone size and time. It would be interesting to know whether growth was linear

indicating that the limiting factors might be substances produced in the urinary tract or whether the relationship was exponential indicating that the size of the calculus was important in relation to the rate of growth.

b. Density of calculi

The results of the limited studies carried out in this project agree with the observation of Milks (1935) that magnesium ammonium phosphate calculi are less dense than the other types but the differences are not great.

c. Number of magnesium ammonium phosphate calculi

The results of this study agree with those of other authors in finding that the number of magnesium ammonium phosphate calculi in the canine urinary tract could vary from a single stone to several hundred. Except for Krabbe (1949), published studies have not stated the proportion of cases containing different numbers of calculi. In this series 10 out of 49 cases had single stones while Krabbe found that 21 out of 30 cases had single calculi. She did not publish the composition of these calculi.

d. Shape and surface texture of magnesium ammonium phosphate calculi

The size of urinary calculi was clearly related to the number present. In cases where there were numerous stones only a small proportion could be of a large size. The shape of the stones also appeared to be influenced by the number in the bladder since single calculi were usually spheroidal and tetrahedral calculi were only found when multiple stones were present.

Most authors who have commented on the shape of the calculi support McCunn's (1947) view that the flattened surfaces and tetrahedral shapes are produced by adjacent stones rubbing together. In this study evidence was found to support the view put forward by Haugh, Lonsdale, Mason and White (1966) that tetrahedra

pack more efficiently into the bladder than do spheres and that spherical calculi tend to become tetrahedra by continued growth rather than by friction. It was also noted that in sections from one calculus the laminar rings were circular towards the centre of the calculus and triangular towards the edge suggesting the shape was due to growth rather than attrition. However this theory of compaction does not conform with the occurrence of the small tetrahedral calculi found in this study and they may be due to orientation of the crystals as suggested by Haugh et al. (1966). A further explanation of tetrahedral calculi might be that they are formed by the fracture of a larger round calculus. There was no direct evidence that this took place in any of the cases in this study, although many of the calculi had radial fissures, seen on radiographs, which, had they been complete, would have divided the stones into roughly tetrahedral fragments.

The shape of the unusual magnesium ammonium phosphate calculi with sharp plate-like projections is probably due to some special type of crystal growth although factors influencing the formation of these calculi could not be determined. Bacteria were not found in these calculi or in urine samples from this dog.

Although fairly large multiple calculi were present in the bladder of a dog with ammonium urate calculi these stones showed no tendency to become tetrahedra.

In the present study there was evidence that the shape of magnesium ammonium phosphate calculi may also be influenced by the shape of the containing organ. This finding is in agreement with White's (1966) observations. However the organ probably only exerts an influence when the calculus becomes large enough to be in continuous contact with the mucous membrane of the wall.

The calculi in this study were shades of grey or white thus resembling those reported by other authors (Blount, 1931; Milks, 1935; Bloom, 1954; White, Treacher and Porter, 1961; Smith and Jones, 1966; Goulden, 1966).

The surface texture appeared to be influenced by the presence of surrounding structures such as other calculi and the organ wall. Surfaces in contact with other calculi were smooth while those in contact with the bladder mucous membrane were rough.

e. Distribution of crystals in calculi

As apatite crystals are more radio-dense than the other types found in calculi, the presence of this substance was detected in a considerable number of sections examined by microradiography, but the amounts were too small to be detected by X-ray diffraction analysis. It was noted that most canine calculi contained more than one crystalline component but only a few combinations occurred. When a calculus contained two or more crystalline constituents, each substance was deposited in distinct areas within the calculus and complete intermixing of crystals throughout the stone did not occur. The canine calculi in this respect resembled human calculi studied by Lagergren (1956).

The combinations of crystals most frequently observed were magnesium ammonium phosphate with apatite, and calcium oxalate monohydrate, calcium oxalate dihydrate and apatite together. The cystine calculi examined consisted only of crystals of this substance but the number of stones was too small to conclude that combinations might not occur. In three calcium oxalate calculi the positions of the monohydrate and dihydrate crystals were determined on the basis of the composition of the calculi and on the greater radio-density of the monohydrate salt. The canine calculi resembled those described by Murphy and Pyrah (1962) where the inner part of the stone consisted of monohydrate crystals while the

outer layer was composed of projecting dihydrate crystals.

f. Nuclei

Study of the literature revealed considerable diversity of opinion on the composition of nuclei. Some authors mentioned bacteria, epithelial cells or a single crystal while others mentioned a much larger structure such as suture material or a small calculus of different composition. It would be difficult to look for a nucleus the size of the former group, but using Murphy and Pyrah's (1962) definition of a "focal point or area which differs in composition from the rest of the stone" nuclei were occasionally seen in canine calculi. This result agrees with the observations of Murphy and Pyrah.

The central areas in two cases of magnesium ammonium phosphate calculi were of particular interest as macroscopic and microradiographic study suggested that they were composed of cystine. As both dogs had previously been treated for cystine calculi it seemed probable that the development of staphylococcal infection allowed magnesium ammonium phosphate to become deposited round small cystine stones.

Although the presence of foreign material acting as a nucleus is mentioned in the literature as an important cause of urolithiasis (Stainton, 1922; Blount, 1931; Milks, 1935; Smith and Jones, 1966), this study did not reveal evidence to support that view.

g. Lamination

Concentrically arranged laminar bands were seen in most of the magnesium ammonium phosphate calculi examined in this study. This agrees with the observations of Bloom (1954), Cornelius and Bishop (1961) and Goulden (1966). Microradiography of thin sections showed that these laminae were probably produced

by alternating layers of apatite and magnesium ammonium phosphate. Lonsdale (1968a) suggested that the laminae in calculi of human origin corresponded to daily or seasonal variations in urinary pH, but this explanation is unlikely to be correct as Prien (1949) observed that apatite could be precipitated over a wide range of urinary pH. The two salts do not precipitate together but form alternating pure layers and some local factors other than pH must act at the surface of the calculus to produce this effect.

Concentric laminae were seen in human calculi composed of calcium oxalate monohydrate (Lagergren, 1956; Murphy and Pyrah, 1962), but they were not observed in three canine stones of this type in this survey. Two canine cystine calculi showed lamination on macroscopic examination but were homogeneous on microradiography. A similar result was observed by Boyce et al. (1958) in human cystine calculi. Marked lamination was also seen in the single canine ammonium urate calculus and this result fitted with the common observation that when these calculi are fractured they break into multiple shells.

h. Radial striation

In this study two magnesium ammonium phosphate calculi showed some radiating lines of darker colour resembling those described by Cornelius and Bishop (1961) and by Goulden (1966). A radial arrangement of crystals was not observed on microradiography and this finding agrees with Murphy and Pyrah (1962) who noted that magnesium ammonium phosphate calculi from human patients did not show radial striations.

Radial striations were found to occur in human calculi composed of calcium oxalate monohydrate, calcium hydrogen phosphate dihydrate and ammonium urate (Carr, 1953; Lagergren, 1956). Such striation was not seen in the small range of canine calculi examined except for the single ammonium urate stone in which

one laminar band exhibited radial striation. One of the calcium oxalate calculi appeared to have a general radial arrangement of the crystals but did not show the dense radiating lines described in human calculi.

i. Column formation

Columns of magnesium ammonium phosphate crystals as described by Lagergren (1956) were seen only in part of one calculus and this finding agreed with Murphy and Pyrah's (1962) observation that in human calculi the columnar form was uncommon. However, six of the canine calculi showed areas where large magnesium ammonium phosphate crystals appeared as loose aggregates without apparent organisation. When these calculi were decrystallized and sectioned, the matrix was found to extend round and between the crystals.

j. Fissures

Although cracks or fissures were only seen in canine calculi consisting of magnesium ammonium phosphate, the sample of canine stones of other composition was insufficient to conclude that fissures do not occur in other types of calculi.

Fissures in canine calculi do not appear to have been recorded previously. The possibility that the fissures were due to contraction associated with drying of the calculi was considered but evidence against this explanation was noted in two cases where fissures were seen in calculi while still within the bladder.

Similar cavities in human calculi have been noted (Prien, 1955; Lagergren, 1956; Boyce et al., 1958), but the method by which they form is unknown. It is likely that in the fresh specimen these fissures are filled with fluid but it is difficult to understand how crystallization could take place round such a cavity or, alternatively, how crystals already deposited would be removed.

5. CONCLUSIONS

The weights of calculi which formed in the canine urinary tract differed according to the sex of the animal and the composition of the calculus. Calculi forming in female dogs were heavier than those forming in male animals, while magnesium ammonium phosphate stones were heavier than calculi containing calcium salts or cystine.

The number, size, shape and surface texture of magnesium ammonium phosphate calculi appeared to be inter-related. The shape and surface texture of the calculi could also be influenced by the shape of the organ in which the stones developed.

Nuclei were not consistently found, or considered to be important in the formation of canine calculi, although in several calculi the central parts of the stones differed from the surrounding parts.

Many of the magnesium ammonium phosphate calculi were formed of concentric layers of crystals. These bands consisted of alternating deposits of magnesium ammonium phosphate and apatite.

Radial striation was not a common feature in the canine calculi examined in this study. Column formation was seen only in a single magnesium ammonium phosphate stone.

Central fissures occurred in several magnesium ammonium phosphate calculi.

PART III Properties of bacteria associated with magnesium ammonium phosphate urolithiasis

In Part I of this study it was noted that bacteria, especially staphylococci, were usually present in the urine of dogs with magnesium ammonium phosphate urolithiasis and cocci were usually found in the matrices of these calculi. These findings indicate that bacteria may play an important part in the formation of magnesium ammonium phosphate stones, so the possible implication of bacterial enzymes was also considered.

In this third part of the investigation it was decided to carry out tests for urease and phosphatase production on the organisms isolated from dogs with urolithiasis and also to try to characterise the staphylococci by their ability to produce coagulase, haemolysis and pigment.

1. REVIEW OF THE LITERATURE

a. Urease

Pasteur and Joubert (1876) noted that microscopic organisms were able to produce ammonium carbonate from urea in human urine resulting in a rise in pH. Stainton (1922) and Krabbe (1949) considered the alteration of pH produced by bacterial action on urea to be an important etiological factor in canine calculi composed of magnesium ammonium phosphate. This theory was also suggested for the same type of calculus in man by Chute (1938).

Goulden (1968) studied infection in dogs with urolithiasis and discussed the action of bacteria on their etiology, concluding that the ability of the bacteria to split urea did not completely account for the formation of stones.

Elliot, Sharp and Lewis (1959) found that the solubility of magnesium ammonium phosphate in urine was determined mainly by the pH and the concentrations of magnesium, ammonium and inorganic phosphate ions. Urea-splitting bacteria can produce an elevation of pH and ammonium ion concentration which reduces solubility and so encourages crystal formation. No information was found in the literature relating to the magnesium concentration in the urine of dogs with magnesium ammonium phosphate calculi, but Elliot, Sharp and Lewis (1959) found no elevation of the magnesium level in human urine with these crystals.

b. Techniques for measuring urease

A qualitative method of detecting urease production by bacteria was described by Christensen (1946) who inoculated the organisms on to a medium containing urea and an indicator. After incubation, a colour change in the medium showed that the urea had been broken down. Cruikshank (1965) also suggested a similar medium for detecting urease activity. White and Hill (1941) criticised techniques involving growth of the organisms on media containing

urea and a pH indicator on the grounds that a base other than ammonia may be produced or ammonia may be formed from a substance other than urea. They also considered that small amounts of ammonia may be inadequate, in the presence of buffers, to alter the pH sufficiently to change the indicator. These authors suggested that the test should involve only urea and bacterial urease and that other organic matter should be excluded.

The urease activity of bacteria was measured by Schulte and Thompson (1941) using a technique where they collected and titrated ammonia produced by bacteria on a urea substrate.

Van Slyke and Archibald (1944) described three biochemical techniques available for measuring urease activity although they did not use bacterial enzyme. The first method involved the collection and measurement of carbon dioxide produced by the breakdown of urea, while the second method involved collection of ammonia and its measurement by titration. The third method was based on the principle that the time required for a given amount of product to form is inversely proportional to the amount of enzyme available. Enzyme was added to a solution containing urea and the time taken for the pH to rise from 6.8 to 7.7 was measured.

This last method involving the time taken for change in pH was used by Carroll and Brennan (1952a) to measure urease activity in bacteria.

Davalos (1943) studied the urea-splitting activity of bacteria in urine by measuring the concentration of urea and phosphates before and after incubation with organisms.

Day, Gibbs, Walker and Jung (1930) studied urease production by Proteus vulgaris and noted that urease was an endocellular enzyme. They found that the rate of hydrolysis of urea increased with increasing concentrations of urea up to

a maximum effect at 2.04% urea in broth, after which no further increase took place. Small numbers of actively growing bacteria could produce a marked breakdown of urea but organisms which were not reproducing hydrolysed urea much more slowly.

c. Urease production by bacteria isolated from animals with urolithiasis

Several workers have tested organisms isolated from the canine urinary tract for urease production by growing the bacteria on a urea medium.

Brodey (1955) tested staphylococci isolated from dogs with calculi and found that 13 out of 15 haemolytic coagulase positive strains split urea while only 8 out of 14 non-haemolytic coagulase negative strains could do so.

Fritsch and Zuylen (1966) carried out urease tests on 10 strains of staphylococci and 4 strains of Proteus finding all strains positive. They also tested 4 strains of Pseudomonas aeruginosa of which 2 were positive and 2 negative, and 6 strains of streptococci finding 2 strains positive and 4 negative. Two strains of Escherichia coli were tested and both failed to split urea.

The urease activity of bacteria isolated from 22 cases of canine urolithiasis was studied by Goulden (1968) who found that all the strains of staphylococci associated with magnesium ammonium phosphate stones were urease positive although a strain associated with a calcium oxalate calculus was urease negative. Strains of streptococci and E. coli isolated from his animals all failed to split urea.

Bacteria isolated from cases of urolithiasis in mink were examined by Nielsen (1956) who found that all 10 strains of Proteus and 36 out of 38 strains of haemolytic staphylococci could split urea.

d. Urease production by bacteria isolated from human patients

More extensive studies on urea-splitting bacteria have been carried out on organisms isolated from human cases of urolithiasis.

Young and Davis (1926) examined 100 strains of staphylococci from various sources and found only 5 which could produce urease. Jolly (1929) also noted that many strains of staphylococci were unable to break down urea, although they appeared to be able to form phosphate calculi.

A series of 100 strains of staphylococci were examined by Earlam (1930) who found that 31 strains could split urea but that there did not appear to be much difference between Staph. aureus, Staph. albus and Staph. citreus in this respect. A similar result was obtained by Eisenstaedt (1931) who found that only 7 out of 23 strains of staphylococci were urease positive.

Hill and White (1941) found that 73 strains of staphylococci from 103 strains tested were able to split urea while Carroll and Brennan (1952b) found 19 positive strains in 39 strains examined. The latter workers failed to find any correlation between urea-splitting action and coagulase or haemolytic activity.

Almost all strains of Proteus examined by Hill and White (1941) and Carroll and Brennan (1952b) were able to produce urease, but while Hill and White (1941) found that 21 out of 58 strains of E. coli could break down urea, Carroll and Brennan (1952b) could find only 1 positive strain in 99 tested.

Urea-splitting streptococci were found only in 1 strain of 33 tested by Hill and White (1941) and no positive strains were found by Carroll and Brennan (1952b) after testing 18 strains of this organism.

Giertz (1946) studied streptococci isolated from human urinary tract infections and found that none of the 208 strains tested could split urea sufficiently to alter the pH of urine. He also noted that he had found no urea-splitting strains of E. coli in 19 cases tested.

e. Coagulase

Cruikshank (1965) stated that most strains of staphylococci isolated from lesions in man and animals could coagulate plasma but coagulase negative strains were occasionally associated with infective conditions. Smith (1947) studied staphylococci of animal origin and confirmed that organisms isolated from pathological lesions could coagulate plasma while those recovered from healthy tissues could not do so. The coagulase test was carried out by Smith (1947) on 39 strains of staphylococci isolated from dogs. He used human and bovine plasma and found 24 strains which produced coagulase but also noted a difference in the rate of coagulation of human and bovine plasma. Using human plasma none of the strains of staphylococci produced coagulation within three hours although all 24 strains were positive overnight, while 20 strains coagulated bovine plasma within three hours and the remaining 4 strains were positive overnight.

f. Coagulase production by staphylococci isolated from animals with urolithiasis

Brodie (1955) isolated 28 strains of staphylococci from dogs with urolithiasis and found that 15 strains were coagulase positive while Goulden (1968) recovered 21 strains of staphylococci from 17 cases of canine urolithiasis and he noted that 18 strains could coagulate rabbit plasma.

Staphylococci isolated from mink with urolithiasis were studied by Nielsen (1956) who tested 26 strains of haemolytic staphylococci and found that 22 of these strains could coagulate human plasma.

g. Phosphatase

Gordon and Marshall (1930) found that certain types of bacteria were able to form ortho-phosphate from glycero-phosphate, and they included Staph. aureus, P. vulgaris and some strains of streptococci in their list. They also observed

that other strains of staphylococci were unable to produce this reaction. Further studies by Gordon and Cooper (1932), using bacteria killed by chloroform, confirmed the presence of glycero-phosphatase in E. coli and staphylococci. Boivin and Mesrobeanu (1933) found that E. coli and Staph. aureus produced two phosphatases which were effective at different pH levels.

Bray and King (1942) described a medium containing phenolphthalein phosphate. If organisms which could produce phosphatase were grown on this medium, free phenolphthalein was liberated and its presence detected as a pink colouration when the medium was exposed to ammonia vapour. A modification of this technique was described by Barber and Kuper (1951) who used a concentration of 0.01% phenolphthalein diphosphate in nutrient agar.

Cordonnier and Millar (1951) produced experimental renal calculi in rabbits and rats but could detect no increase in alkaline phosphatase in the kidneys of those animals which formed stones.

According to White, Handler and Smith (1959) phosphatase increases the breakdown of organic phosphate compounds to inorganic phosphates and this enzyme is found in high concentrations where calcium phosphate is being deposited in developing bone. Oser (1965) noted that of the total phosphate content of urine, organic phosphate forms only 1 - 4%.

h. Phosphatase production by bacteria

No reference was found to studies on phosphatase activity in bacteria of canine origin but Bray and King (1943) found positive reactions with staphylococci and some strains of streptococci from human sources while they also obtained weakly positive reactions with E. coli. These workers did not test the phosphatase activity of Proteus.

Barber, Brooksbank and Kuper (1951) found a close correlation between

coagulase and phosphatase production by staphylococci. Using organisms isolated from human sources they found that of 160 coagulase positive strains all produced a positive phosphatase reaction within 24 hours while of 75 coagulase negative strains which they tested only 1 produced phosphatase within 24 hours although a further 54 strains were positive after 2 - 7 days.

The phosphatase activity of Gram negative bacteria was studied by Cowan and Steel (1965) who found positive strains of Proteus species and Escherichia species.

i. Haemolysis

The haemolytic action on sheep blood of staphylococci isolated from dogs was studied by Smith (1947) who concluded that haemolysis was not related to coagulase production. However, his results did seem to suggest that coagulase and haemolytic activity were frequently found together as all 24 coagulase positive strains were haemolytic while only 3 out of 15 coagulase negative strains produced haemolysis.

Brodey (1955) mentioned the haemolytic ability of staphylococci isolated from dogs with urolithiasis and while he did not give much information on this point he seemed to claim that all coagulase positive strains of staphylococci were haemolytic and all the coagulase negative strains were not.

Fifty-two strains of staphylococci were isolated from mink with urolithiasis by Nielsen (1956) who used beef blood medium to separate 37 haemolytic strains from 15 non-haemolytic strains. He used only haemolytic strains for coagulase tests and found that 22 out of 26 haemolytic strains of staphylococci were also coagulase positive.

Cowan and Steel (1965) noted that differing degrees of haemolysis could be produced by staphylococci in blood from differing species and they suggested

sheep blood as most satisfactory for demonstrating haemolysis by these organisms.

Cruikshank (1965) stated that most coagulase positive strains of staphylococci produced haemolysis in rabbit or sheep blood but that little or no haemolysis was produced using horse blood.

Using sheep and rabbit blood Elek and Levy (1950) found that three haemolysins were associated with coagulase-producing staphylococci isolated from man and animals. They also found that a high proportion of coagulase negative strains could produce haemolysis.

j. Pigment production by staphylococci

Smith (1947) studied pigment production by staphylococci isolated from dogs and found that golden pigment was produced by 3 out of 24 coagulase positive and by 5 out of 15 coagulase negative strains. He also observed that this pigment only appeared after incubation for 72 hours.

2. MATERIALS AND METHODS

a. Bacteria studied

The bacteria examined in this study were isolated from the urine of dogs with urolithiasis. In several cases differences in the appearance of the colonies or in the ability to haemolyse horse blood suggested that two strains of the same organism were present. When this occurred these strains were tested separately. When dogs developed urolithiasis on more than one occasion the bacteria isolated at each episode were included. Twenty-seven strains of staphylococci were obtained from 24 episodes of magnesium ammonium phosphate urolithiasis involving 21 dogs and, for comparison, study was made of a further 7 strains from 6 dogs which had either calculi of a different composition or had no stones at this time but had been treated for magnesium ammonium phosphate calculi on a previous occasion. Four strains of streptococci, 3 strains of P. mirabilis and 2 strains of E. coli were isolated from 7 dogs with magnesium ammonium phosphate urolithiasis.

b. Urease test

All the organisms were tested for urease production using the technique described by Cruikshank (1965). Bacteria were inoculated on to slopes of Christensen's medium and incubated at 37°C. Production of urease was detected by the development of a dark pink colour in the media. If no colour change occurred after incubation for six days it was concluded that the organism could not produce urease.

c. Coagulase test

The coagulase test was carried out on all strains of staphylococci using the tube method and rabbit plasma as described by Cruikshank (1965). Overnight

broth cultures of staphylococci were added to rabbit plasma diluted 1 in 10 in normal saline and the plasma was incubated at 37°C for 6 hours and then left at room temperature overnight. The tubes were examined for coagulation of the plasma at the end of the fourth and twenty-fourth hours. Organisms which produced clots within 24 hours were considered to be coagulase positive.

d. Phosphatase test

All the strains of bacteria were tested for phosphatase production using phenolphthalein phosphate agar as described by Cowan and Steel (1965). After overnight incubation cultures were exposed to ammonia vapour to detect free phenolphthalein, indicating colonies of organisms which had produced phosphatase.

e. Haemolysis test

Staphylococci were tested for their ability to produce haemolysis. Horse blood agar plates were inoculated with urine and incubated at 37°C for 24 hours. If different types of colonies developed representative colonies were sub-cultured on to horse blood agar and the presence or absence of haemolysis noted after incubation for 24 hours. All strains of staphylococci were also tested for the ability to haemolyse sheep blood by sub-culture on to sheep blood agar plates.

f. Pigment production test

Broth cultures of staphylococci were sub-cultured on to 10% milk agar, incubated at 37°C for 18 hours and then exposed to light at room temperature for 14 days. The cultures were examined daily.

3. RESULTS

The source of the organisms studied and the detailed results of the tests carried out on them are given in Appendix 7.

a. Urease

Forty-three strains of bacteria were examined for urease production and the results are summarised in Table 28.

Table 28

Urease production

Type of organism	Number of strains tested	Urease positive
Staphylococci	34	33
Streptococci	4	0
P. mirabilis	3	3
E. coli	2	0

b. Coagulase

Thirty-four strains of staphylococci were tested for coagulase production and 31 strains were able to coagulate plasma. Only 2 dogs were infected with coagulase negative staphylococci as organisms were isolated on 2 separate occasions from case 42A. Twenty strains were able to coagulate rabbit plasma within 4 hours while a further 11 strains produced coagulation within 24 hours.

c. Phosphatase

Forty-three strains of bacteria were tested for phosphatase production and the results are summarised in Table 29.

Table 29

Phosphatase production

Type of organism	Number of strains tested	Phosphatase positive
Staphylococci	34	33
Streptococci	4	0
P. mirabilis	3	0
E. coli	2	0

The only phosphatase negative strain of staphylococci was isolated from case 10 and the organisms were also unable to produce coagulase or urease.

Three additional strains of Proteus isolated from the urine of dogs without calculi were tested and 2 of these strains gave weakly positive reactions.

d. Haemolysis

Thirty-four strains of staphylococci were tested for their ability to cause haemolysis when grown on horse and sheep blood agar plates and the results are summarised in Table 30.

Table 30

Haemolysis of horse and sheep blood by staphylococci

	Horse blood	Sheep blood
Number of strains tested	34	34
Haemolytic	8	29
Non-haemolytic	26	5

e. Pigment production

Only 2 strains of staphylococci out of 34 strains tested were able to produce golden pigment. In one case the pigment developed within 24 hours while in the other it formed within 48 hours.

4. DISCUSSION

The results of this study showed that almost all strains of staphylococci isolated from dogs with magnesium ammonium phosphate calculi were able to produce urease. This finding is in agreement with the results published by Brodey (1955) Fritsch and Zuylen (1966) and Goulden (1968).

A limited number of other organisms associated with magnesium ammonium phosphate stones were examined and it was found that P. mirabilis could produce urease but streptococci and E. coli could not. These observations were also in accordance with the results of other workers except Fritsch and Zuylen (1966) who isolated two urease-producing strains of streptococci.

A high proportion of staphylococci isolated in this study were able to coagulate plasma and in this respect they conform with the results published by Goulden (1968) but differ from studies carried out by Brodey (1955) who found that only about 50% of the strains which he isolated were coagulase positive.

All staphylococci tested could produce phosphatase apart from one strain. This conforms with the report by Barber, Brooksbank and Kuper (1951) who found that coagulase positive staphylococci from human sources usually produced phosphatase. Coagulase negative strains of staphylococci were isolated on only three occasions in this study. Two strains were able to produce phosphatase but the third strain could not do so.

Phosphatase was not produced by strains of P. mirabilis, streptococci or E. coli isolated from dogs with magnesium ammonium phosphate calculi but, when a further three strains of Proteus isolated from the canine urinary tract were tested, weak positive reactions were obtained in two cases indicating that some strains of this organism can break down organic phosphates. Haemolysis of sheep red blood cells was produced by most of the staphylococci but only a few strains

were able to haemolyse horse blood. A similar result was recorded by Cruikshank (1965). The ability of staphylococci to produce coagulase did not appear to be closely related to haemolytic activity and this agrees with Smith's (1947) observation.

Golden pigment was produced by only two strains of staphylococci, the remainder producing white colonies. The golden pigment was seen within 48 hours as compared with Smith (1947) who found that over 72 hours' incubation was necessary.

Staphylococci isolated from dogs with magnesium ammonium phosphate urolithiasis were usually able to produce urease, coagulase, phosphatase and haemolysis of sheep blood but none of these properties were invariably present. Comparison of these organisms with a small number of staphylococci from the urine of dogs affected with other conditions failed to find any important difference between the two groups.

Staphylococci appeared to be able to reduce the solubility of magnesium ammonium phosphate by the effect of urease on pH and the concentration of ammonium ions, and also by the effect of phosphatase in increasing the concentration of inorganic phosphate ions. However, the amount of organic phosphate in urine recorded by Oser (1965) would appear to be too low to provide sufficient phosphate ions to affect solubility.

5. CONCLUSIONS

Urease was produced by 33 out of 34 strains of staphylococci and by all 3 strains of *Proteus* tested. Four strains of streptococci and 2 strains of *E. coli* were all urease negative.

Coagulase was produced by 31 out of 34 strains of staphylococci while phosphatase was produced by 33 strains of this organism. Four strains of streptococci, 3 strains of *Proteus* and 2 strains of *E. coli* were unable to produce this enzyme.

Thirty-four strains of staphylococci were tested for the ability to cause haemolysis. Sheep blood was haemolysed by 29 strains and horse blood by 8 strains. Two strains of staphylococci produced golden pigment and 32 strains white colonies.

Urease and phosphatase produced by bacteria could influence the solubility of magnesium ammonium phosphate but neither enzyme appeared to be essential in calculus formation.

PART IV Experimental studies on urolithiasis

Research on urolithiasis has been hampered by the absence of a reliable experimental procedure for producing calculi. Attempts have been made to grow calculi in human urine *in vitro* with only limited success but no such studies appear to have been carried out using dog urine. It was decided to investigate the growth of magnesium ammonium phosphate calculi in sterile dog urine and in urine infected with the organisms most frequently associated with this type of calculus. If calculi could be produced *in vitro* resembling the naturally formed stones, then all essential factors must be present in the urine and the urinary tract must be relatively unimportant in their formation. The search for the cause of urolithiasis would then be made considerably easier.

Although the incidence of urolithiasis is relatively high in the canine species, dogs have rarely been used as experimental animals in the study of this disease. There appear to be marked differences in the incidence and the etiology of urolithiasis in different species and the results of studies on laboratory animals do not appear to be directly applicable to dogs. This section describes the procedures used in an endeavour to produce calculi experimentally in dogs. The effect of staphylococci and *Proteus* organisms on crystal precipitation, stone growth and urinary pH was also studied *in vitro*.

1. REVIEW OF THE LITERATURE

a. Production of calculi in vitro

Pasteur and Joubert (1876) noted that bacteria could multiply in human urine and that some types of bacteria could produce a rise in pH by forming ammonia from urea. Cox and Hinman (1961) found that Escherichia coli multiplied at similar rates in urine and broth but Asscher, Sussman, Waters, Davis and Chick (1966) found that the growth of E. coli in urine was affected by the osmolarity of the urine. They also found that growth did not take place at a pH lower than 5.5, or, in 7 of 10 strains tested, at a pH above 7.5. The effect of pH on bacterial growth in canine urine does not appear to have been investigated but Davis and Hain (1918) noticed that some urine samples from normal dogs inhibited the growth of E. coli and Staphylococcus aureus. Hain (1920) showed that the antibacterial action appeared to be unrelated to the urinary pH.

The growth of bacteria in human urine was studied by Pillet (1927) who found that when sterile filtered acid urine was inoculated with staphylococci it became alkaline and numerous magnesium ammonium phosphate crystals were precipitated. Eisenstaedt (1931) obtained the same results with staphylococci but also showed that some strains of E. coli brought about a rise in pH and the precipitation of crystals while Davalos (1943) observed that, after inoculation with Proteus organisms, magnesium ammonium phosphate crystals were precipitated and the concentrations of urea and phosphate in urine decreased.

King and Boyce (1963) recorded the results of a series of experiments on the growth of calculi in human urine. They exposed a renal calculus for three weeks to a continuous flow of urine from patients who had urolithiasis and found that, although there was bacterial growth and precipitation of salts, the calculus did not increase. They then exposed two calculi to urine from normal subjects,

changing the urine daily for 11 weeks. In this experiment they found that the magnesium ammonium phosphate/calcium phosphate calculus decreased from 1.45g to 1.27g and the calcium oxalate calculus decreased from 0.81g to 0.77g. King and Boyce described a procedure for simulating the growth of magnesium ammonium phosphate/calcium phosphate calculi by placing silk suture material in 50 ml of normal urine which was changed daily and supplemented with 400 mg of calcium per litre of urine. Bacterial contamination of the urine occurred and Proteus mirabilis, Pseudominas aeruginosa, Aerobacter aerogenes, E. coli, streptococci and paracolon organisms were isolated. The composition of the mineral and organic components of these concretions resembled those found in naturally occurring calculi in man; however, the artificial concretions did not resemble the shape of natural calculi.

Barnhouse (1968), using a technique similar to that of King and Boyce (1963), attempted to prevent bacterial contamination of the urine by the use of more elaborate apparatus and by filtering urine through 0.6 μm porcelain filters or 0.22 μm millipore filters. He used 40 ml of human urine, changing these samples every day for 5 days per week and allowing each experiment to run 7 weeks. Samples were inoculated with cultures of Ps. aeruginosa, E. coli, or P. mirabilis which had been obtained from the urine of human patients with calculi. He noted that only P. mirabilis infection increased the urinary pH and he concluded that the bacteria he tested did not directly affect crystallization in urine.

A slightly different technique for producing artificial calculi was employed by Vermeulen, Lyon and Gill (1964) who were able to obtain a crystalline deposit on a wire stirrer immersed in urine to which additional ions were added by means of filter paper wicks. These authors claimed to produce uric acid, oxalate and phosphate calculi by adding the appropriate ions to urine. In a further paper

Lyon and Vermeulen (1966) produced calcium oxalate crystallization in a synthetic medium similar to urine, while Vermeulen, Ellis and Hsu (1966) concluded that the mechanisms of urinary calculus formation could be explained as a crystallization process from a supersaturated solution. The latter authors observed that the artificial concretions often assumed many of the structural characteristics of natural calculi.

Boyce (1969), summing up research on stone growth in human urine, said "Attempts to simulate concrement formation in crystal growth chambers ... have failed to produce morphologically acceptable models of human concretions from any urine or solutions which even closely resemble those encountered in vivo". He observed that his attempts to grow calculi had produced a fragile collection of crystal precipitates on the most acceptable interface for precipitation.

Thomas, Bird and Tomita (1963) noted that urine from most human patients with renal calculi could mineralise rachitic rat cartilage matrix in vitro, while urine from most normal subjects could not. They used this test to study factors in urine which might influence stone growth. They found no differences in the concentrations of urea, sodium, titratable organic acids, magnesium or calcium between mineralising and non-mineralising urine, but incubation of urine with P. vulgaris would convert normal non-mineralising urine to the mineralising form.

b. Experimental urolithiasis in dogs

Deposits of salts were obtained on foreign bodies implanted in the renal pelves of 7 dogs by Collica (1948) while Edwards, Garvey and Boyce (1963) studied the growth of calculi on human stone fragments and silk sutures implanted in pouches of dog bladder, some in contact with urine and others isolated from the urine. In the experiments using stone fragments they found crystals deposited on 3 fragments in contact with urine and no enlargement of 7 fragments isolated

from urine, while stones formed on 11 out of 14 silk sutures in contact with urine but not on 22 sutures isolated from urine. Bacteriological studies were not carried out but all the dogs with foreign body stones showed evidence of pyelonephritis and Edwards, Garvey and Boyce (1963) considered that unless the animals could be maintained in a germ-free environment it might be impossible to evaluate the effect of bacteria on experimental calculosis. In their studies the stones formed over periods ranging from 2 to 17 months.

Mani and Scott (1967) studied the formation of calculi around braided sutures composed of a mixture of teflon and dacron, also around braided silk, placed in the bladders of dogs and cats. They infected some of the dogs by instilling a broth culture of Proteus into the bladder. All the animals were destroyed after three months. There was no evidence of stone formation in cats, but calculi had formed round both types of suture material in dogs. Analysis of the calculous material showed it to be composed of magnesium ammonium phosphate and calcium phosphate. Mani and Scott (1967) found no significant difference in the incidence of stones between the animals which had been infected with Proteus and the others, although they thought that infection enhanced the rate of stone growth. Unfortunately, these authors did not publish the results of bacterial examination of the urine at the end of the experiment and it is not clear whether the infection persisted or not. Yudofsky and Scott (1969) found that crystal precipitation occurred on a variety of suture materials placed in the bladders of dogs but they did not test braided nylon suture.

Several research workers have tried to establish chronic urinary infection in dogs in order to study conditions other than urolithiasis. Hueper, Fisher, Carvajal-Forero and Thompson (1941) irradiated the bladders of 5 dogs with X-rays and found that infections developed which in some cases persisted for over three

months. They mentioned Staph. albus as one of the invading organisms but did not record any development of calculi.

Scott (1964) resected the mucosal flap of the vesico-ureteral junction in 4 female dogs and after this operation infection with Staph. albus developed in 2 animals and persisted throughout the 21 months of the experiment but Scott made no mention of calculi developing. He also tried to infect these dogs with cultures of E. coli and Staph. aureus but these infections did not persist.

Cultures of P. vulgaris on nutrient agar were implanted under the mucous membrane of the bladders of 16 dogs by Schoenberg, Beisswanger, Howard, Walter and Murphy (1965) but this technique failed to produce urinary infection. They repeated the procedure and crushed a small area of bladder muscle which resulted in bacteruria developing in 20 out of 23 dogs. Infection was still present six months later in 16 animals. At autopsy these dogs showed no calculi and no pyelonephritis.

Sommer and Roberts (1966) injected 5 ml of paraffin wax at 60°C via a catheter into the bladder of 27 dogs and 8 of these were experimentally infected by the infusion of broth cultures of E. coli while 17 were infected with P. mirabilis. These workers found that the paraffin wax rarely became encrusted with salts in the crevices of the paraffin despite the prolonged presence of infection and they considered the non-wettable surface of the paraffin inhibited deposit of this nature.

Magnesium ammonium phosphate calculi developed in mink when Nielsen (1956) infected the urinary tract either by instilling a culture of staphylococci into the bladder via the urethra or by an injection into the ureter at laparotomy. He was able to produce stones in 7 out of 15 cases where bacteria had been placed in the untraumatised bladder; he also produced calculi when the organisms had

been placed in the ureter, while animals treated in the same way but without bacteria did not develop stones.

Hryntschak (1934) reported that calculi developed in 9 out of 13 rabbits after the intravenous injection of staphylococci and partial ligation of one ureter, but no calculi developed in 6 similar cases in which a variety of other organisms were used.

Suby and Suby (1947) isolated a variety of bacteria from human cases of urolithiasis. They did not publish details of these organisms but found that when they were injected into the bladder or intravenously in normal rabbits infection lasted only a short time. After temporary ligation of one ureter they found that intravenous injection of organisms produced prolonged urinary infection with stone formation in the renal pelvis on the ligated side.

Bladder infection with P. mirabilis was investigated in guinea pigs by Sunshine (1964); he also found that after injecting these organisms into the normal bladder the infection which resulted was rapidly eliminated. Infection persisted when a plastic bead was sutured to the mucous membrane of the bladder but no calculi were recorded despite the presence of this foreign body and infection with urea-splitting organisms.

Gregory, Wein, Sansone and Murphy (1971) studied the resistance of the canine bladder to infection. They devised a technique for continuous infusion of broth cultures of bacteria into the bladders of dogs for periods of 2 to 10 weeks. They found no bacteria in urine samples 5 days after discontinuing the infusion.

Cox and Hinman (1961), using broth cultures of E. coli in human volunteers, found that there was a rapid decrease in the numbers of bacteria 6 to 9 hours after the culture had been placed in the bladder and organisms were not isolated

after 72 hours. They showed that urine was not antibacterial as organisms multiplied in broth and urine at a similar rate but the bladder contained an antibacterial factor as organisms multiplied less rapidly in urine in the bladder than in urine *in vitro*.

Studies by Norden, Green and Kass (1968) using radio-isotope-labelled organisms in guinea-pigs showed that by voiding urine 99.9% of the bacteria present in the bladder were removed, but the remaining organisms were sufficient to perpetuate infection if growth was uninhibited. These workers were unable to determine the mechanism by which the bladder can kill bacteria but concluded that it was not due to antibacterial activity of urine, by clumping of organisms on the bladder mucosa, by phagocytosis by leucocytes or related to serum antibody levels.

Variations in the numbers of bacteria present in urine samples from human patients with bladder infection were noted by Friedman and Gladstone (1971). More organisms were present in urine retained overnight than in samples obtained during the day and they considered that a reduced urine flow rate and less frequent bladder emptying could account for this variation.

c. Urinary pH in urinary tract infection and urolithiasis

Fritsch and Zuylen (1966) published detailed results of their examinations of dogs with urolithiasis. From their figures it was possible for the author of the present study to calculate that the mean urinary pH of 15 cases with staphylococcal infection was 7.8 while the mean value for 6 cases with sterile urine was 6.6. In some cases staphylococci were present as mixed infections.

The pH of urine from cases of canine urolithiasis was studied by Goulden (1966) who found that the urine from dogs with magnesium ammonium phosphate stones was significantly more alkaline than that from cases with calcium oxalate calculi.

He found that the mean pH of urine from 17 cases infected with urea-splitting organisms was 7.6 and that this value was significantly greater than the mean pH of 6.7 found in 28 cases where no organisms were isolated. The pH of urine infected with organisms unable to produce urease was not significantly different from uninfected urine.

The effect of pH on the crystallization of stone-forming salts in human urine was studied by Elliot, Quade, Sharp and Lewis (1958) and by Elliot, Sharp and Lewis (1959) who found magnesium ammonium phosphate crystals only in urine samples where the pH was greater than 7.2.

Robertson and Nordin (1969) found that urine from human patients with urolithiasis and urinary infection was oversaturated with magnesium ammonium phosphate due to the high urinary pH and ammonium ion concentration while urine from control patients was undersaturated.

2. MATERIALS AND METHODS

a. Collection and handling of urine samples

Urine samples were obtained in the morning from a male mongrel dog which had no history or radiological evidence of urolithiasis. The urine was collected in a clean container during voiding and stored at $4^{o}C$ for periods up to six hours. The samples were sterilised by Seitz filtration (E.K. filter) immediately before use.

b. Calculi

Four magnesium ammonium phosphate calculi obtained from case 34 were weighed and put in each of 4 x 100 ml rubber-capped bottles. Weighed pieces of braided nylon suture were also introduced into each bottle. The bottles and contents were then sterilised by autoclaving at $130^{o}C$ for four minutes after which they were sealed with the rubber caps.

c. Organisms used to inoculate urine

Strains of organisms isolated from clinical cases of magnesium ammonium phosphate urolithiasis were used for these experiments. Twenty-four hour nutrient broth cultures of Staphylococcus aureus from case 34 and Proteus mirabilis from case 29 were prepared and 1 ml amounts of each of these cultures were used to infect the urine.

d. Experimental procedure for the growth of calculi in urine

The experiments were arranged as described in Table 31.

Table 31

Experimental procedure for the growth of calculi in canine urine

Bottle Number	Urine and bacterial culture added to stone samples and sutures
1	20 ml sterile urine plus 1 ml sterile nutrient broth
2	20 ml sterile urine plus 1 ml broth culture of *Staphylococcus aureus*
3	20 ml sterile urine plus 1 ml broth culture of *Proteus mirabilis*
4	Control sample

After sterilisation the stone and suture from bottle 4 were weighed and the composition of the calculus was checked by X-ray crystallography. To bottles 1, 2 and 3 were added 20 ml of sterile urine. They were incubated at 37°C and at 3 or 4 day intervals the caps of the bottles were disinfected with chlorhexidene in 70% alcohol in water and the urine withdrawn using sterile needles and syringes and replaced with 20 ml of fresh sterile urine. The experiment was continued for 50 days, the bacterial contents of the bottles being checked by cultural and microscopic examination on days 5, 8, 15, 22, 29, 43 and 50 after the start of the procedure. The pH of each urine sample was measured with a pH meter before and after being used.

Twice staphylococci were isolated from the control sample in bottle 1 and both times 50 mg chloramphenicol was added to control this infection. On two occasions *P. mirabilis* in bottle 3 died out and further 1 ml amounts of fresh culture were added.

At the end of the experiment the urine was removed and the contents of the bottles were allowed to dry at room temperature and then weighed.

e. Experimental animals

Seven female dogs, A to G, were used in this study. Dog A was a Beagle which was used only for preliminary tests to find a method of establishing bladder infection. The other 6 dogs were Border Collies. The ages of the animals were unknown but from their appearance and dental development they were judged to be young adults. The animals were kept indoors in individual kennels but were allowed to exercise together in an outside run for periods totalling two hours daily. They were fed on dog biscuit and tinned dog meat and had access to water at all times.

Each dog was subjected to radiographic examination to confirm the absence of urinary calculi and to bacteriological examination of urine to ascertain freedom from urinary infection. Tests were also carried out on urine for the presence of protein, blood and casts and the blood urea level was measured to ensure that these values were within the normal range.

f. Organisms used for experimental infection

The strain of staphylococcus used to infect these animals was obtained from a Spaniel dog with magnesium ammonium phosphate urolithiasis. The organisms were able to produce urease, coagulase and phosphatase. In one case 5 ml of 24 hour broth culture was used and in two experiments 5 ml samples of heparinised blood were collected aseptically from the animals and inoculated with staphylococci. After incubation for 24 hours the blood clotted due to coagulase production by the staphylococci and was used to infect the animals.

g. Technique used to produce persistent bladder infection

Under general anaesthesia and using aseptic techniques laparotomies and cystotomies were performed on the dogs. A piece of heavy braided nylon suture

was passed through the full thickness of the ventral bladder wall and tied with the knot projecting into the lumen of the bladder. A small piece of stainless steel wire was incorporated into the knot to serve as a marker for radiological examination. Infection was produced in three dogs by adding broth or blood clot cultures of staphylococci to the bladder before closing it with catgut sutures. The same procedure, without introducing infection, was carried out in three control animals.

h. Sampling procedures

Urine samples were taken at intervals of several days for the first two weeks to confirm that bladder infection had become established; thereafter, checks were made at periods of approximately eight weeks. On each of the eight week checks the dogs were anaesthetised with a short acting barbiturate and lateral radiographs of the bladder were taken. Urine samples were obtained by paracentesis of the bladder after aseptic preparation of the skin of the posterior abdomen. In cases where the bladder contained only a little urine, samples were obtained by urethral catheterisation. Bacteriological examinations and bacterial counts on these samples were carried out as described for clinical cases in Part I of this thesis. Urinary pH was measured using narrow range pH papers and comparing the colour changes with standard charts.

At the conclusion of the experiments the dogs were destroyed and autopsy carried out. In each case the entire urinary tract was removed and a radiograph taken before the organs were opened and examined macroscopically for the presence of calculi.

3. RESULTS

a. Growth of calculi in urine

The sterilisation procedure produced little effect on the weight of the calculus and suture in bottle 4 (Table 32).

Table 32

Effect of steam sterilisation on calculus and suture

Weight in mg

	Before	After	Change
Calculus	224.5	217.7	Loss 6.8 (3%)
Suture	14.9	15.2	Gain 0.3 (2%)

X-ray crystallographic analysis of the calculus showed no change in the crystalline constituents after sterilisation (Figs. 41, 42).

The changes in weight of samples 1, 2 and 3 at the end of the experiment are given in Table 33.

Table 33

Effect of incubating calculus and suture in sterile and infected urine

Sample	Calculus before	Calculus after	Change	Deposit on suture	Other deposit
1 Sterile	91.7	28.8	-62.9 (68%)	0	0
2 Staphylococci	76.5	80.7	+ 4.2 (5%)	10.8	159.4
3 Proteus	93.2	91.1	- 2.1 (2%)	4.1	72.3

There was a marked loss of weight of the calculus in the sterile urine but only small changes in the calculi in the infected urine. Crystalline deposit formed on the sutures and on the glass walls of the bottles containing infected urine but was absent from sterile urine. The weight of this deposit is also given in Table 33. X-ray crystallographic analysis of the deposit showed that it was composed mainly of magnesium ammonium phosphate and the pattern closely resembled tracings obtained from clinical calculi of this type (Fig. 43).

The sutures, calculi and deposit at the end of the experiments are shown in Fig. 44.

b. Effect of bacteria on urinary pH in vitro

The effect of Seitz filtration on urinary pH was measured on 5 occasions when 60 - 100 ml of urine were filtered and pH increases ranging from 0.05 to 0.15 units were recorded. These changes did not affect the experiments.

The changes in the pH of urine which took place during the experiments are recorded in Table 34.

Table 34

pH of urine before and after incubation with bacteria

Sample	Before	After		
		Sterile	Staphylococci	Proteus
1	6.60	6.55	8.60	9.20
2	6.75	6.75	8.75	7.95
3	5.95	6.25	8.70	9.05
4	7.00	6.85	8.75	9.20
5	5.95	7.35	8.60	9.10
6	6.65	7.05	8.45	9.00
7	6.05	6.55	9.20	9.30
8	6.90	6.70	8.60	9.20
9	6.05	6.40	9.05	9.25
10	7.60	7.20	8.80	9.40
11	5.95	6.40	8.90	9.05
12	6.80	7.60	8.70	9.10
13	6.50	7.50	8.85	9.25
14	6.95	6.55	8.20	9.22
15	5.80	6.30	8.60	9.05

Chloramphenicol (50 mg) was added to the 7th and 12th samples used in the sterile control as staphylococci had contaminated this bottle. One ml of fresh culture of P. mirabilis was added to the bottle containing Proteus with the 2nd and 7th samples.

A marked rise in pH occurred in urine infected with staphylococci and Proteus while the control samples did not change much except when accidental

infection occurred. The mean pH levels of the urine in these experiments are shown in Table 35.

Table 35

Means and standard deviations of pH levels in sterile and infected urine

	Mean	S.D.
Initial urine	6.50	± 0.52
Sterile	6.80	± 0.44
Staphylococci	8.71	± 0.24
Proteus	9.01	± 0.34

Statistical analysis of these results showed no significant difference in urine pH between the initial sample and the sterile control. ($t = 1.67$, d.f. 28, $p > 0.05$). There was a significant difference between the control and the urine infected with staphylococci ($t = 14.69$, d.f. 28, $p < 0.001$) and between the control and the urine infected with *Proteus* organisms ($t = 15.78$, d.f. 28, $p < 0.001$). The sample infected with *Proteus* organisms also reached a significantly higher pH than the sample infected with staphylococci ($t = 2.83$, d.f. 28, $p < 0.01$).

c. Experimental bladder infection in dogs

The detailed results of experimental bladder infection in dogs are given in Appendix 8 and summarised in Table 36.

Table 36

Experimental bladder infection and urolithiasis in dogs

Dog	Organism used	Duration (months)	Calculi produced
C	Staphylococcus	7	Yes
F	Staphylococcus	12	No
G	Staphylococcus	12	No
B	Sterile	12	No
D	Sterile	12	No
E	Sterile	12	No

A calculus formed in the bladder of 1 out of 3 dogs infected with staphylococci while 3 uninfected control dogs did not form stones. X-ray crystallographic analysis of the experimentally produced calculus (Fig. 45) confirmed that it was mainly composed of magnesium ammonium phosphate. Photographs taken at autopsy of dogs C and G show the bladders of these animals and the calculus from dog C (Figs. 46, 47).

At the end of these experiments the staphylococci were re-tested for ability to produce urease, coagulase and phosphatase. The results are given in Table 37.

Table 37

Urease, coagulase and phosphatase production by staphylococci from dogs with experimental bladder infection

Dog	Urease	Coagulase	Phosphatase
C	+	+	+
F	+	-	+
G	-	-	+

The organisms used to infect the dogs were urease, coagulase and phosphatase positive but at the end of the experiments different strains of the organism were isolated from dogs F and G.

d. Urinary pH in experimental bladder infection

The pH of urine samples obtained from the experimental dogs was measured. Only samples obtained after nylon sutures had been placed in the bladders were used. Twenty samples were taken from the three dogs with sterile urine and 20 from the three with staphylococcal infection. The detailed results are given in Appendix 9 and summarised in Table 38.

Table 38

Means and standard deviations of pH levels in urine from experimental dogs with and without infection

	Sterile samples	Infected samples
Number examined	20	20
Mean pH	7.36	7.88
S.D.	$\pm$ 1.08	$\pm$ 0.76

Statistical analysis failed to show a significant difference between the mean pH of sterile urine and urine infected with staphylococci ($t = 1.76$, d.f. 38, $p > 0.05$).

4. DISCUSSION

a. Production of calculi in vitro

Staphylococci multiplied readily in dog urine but Proteus organisms failed to survive longer than a few days. Asscher et al. (1966) found that the growth of E. coli was inhibited by an alkaline pH and the high pH level recorded in the present experiments may explain the elimination of the Proteus infection.

In this study Proteus organisms and staphylococci produced a marked rise in the pH of urine, a result similar to those produced by inoculation of human urine (Pillet, 1927; Eisenstaedt, 1931; Davalos, 1943). The highest pH levels were obtained in urine inoculated with Proteus while the pH of the control urine samples varied little after incubation apart from two occasions when accidental infection occurred.

The marked dissolution of the calculus placed in non-infected urine supports the observation made by King and Boyce (1963) that calculi dissolve in sterile human urine and this indicates that at normal pH levels canine urine is undersaturated with magnesium ammonium phosphate.

King and Boyce (1963) and Vermeulen, Lyon and Gill (1964) were able to produce concretions in human urine by adding certain ions. In the present experiments little change occurred in the calculi placed in infected urine but a considerable amount of crystalline deposit was produced on the suture and the walls of the glass bottles. The crystalline deposit and the fact that the calculi did not lose weight suggest that the presence of bacteria resulted in supersaturation of the urine with magnesium ammonium phosphate. The poor growth of the calculi suggests that factors other than those studied in the experiments may be involved in stone formation. The results of attempted

in vitro production of calculi agree with Boyce's (1969) assessment that the concretions grown in urine are crystalline deposits whose structure does not resemble naturally occurring calculi.

b. Experimental urolithiasis in dogs

The difficulty in producing persistent bladder infection, noted by Gregory *et al.* (1971) was overcome by placing a piece of suture material in the bladder using a technique similar to that employed in guinea pigs by Sunshine (1961).

In the present study calculi failed to grow readily on the suture material and this result conflicts with the findings of Edwards, Garvey and Boyce (1963), Mani and Scott (1967) and Yudofsky and Scott (1969).

Bacterial counts carried out on urine samples from dogs with experimental bladder infection showed quite marked variation between samples from the same animal. Variations in urine flow and bladder emptying are considered to be responsible for similar findings in human patients (Friedman and Gladstone, 1971) and it may be that these factors also account for the variations found in bacterial counts in canine urine.

In the past calculi have not been recorded in the urinary tract of dogs experimentally infected with staphylococci (Hueper *et al.*, 1941; Scott, 1964) or *Proteus* (Schoenberg *et al.*, 1965; Sommer and Roberts, 1966). In the present study a calculus formed round the suture material in one dog infected with staphylococci but two other animals failed to produce calculi. The organisms isolated from the latter animals differed from the staphylococci used to infect them and the changes in the properties of the bacteria may account for the failure of calculus formation. The number of dogs used in these experiments was limited by the accommodation available and by the cost of keeping animals for prolonged

periods.

c. Urinary pH in experimental bladder infection

The urine samples from dogs with experimental infection and from uninfected control animals showed variation over a wide range of pH values.

Although dogs with infection produced urine with a higher pH than the uninfected controls, the difference was not statistically significant. Staphylococci isolated from one dog at the conclusion of the experiments were unable to produce urease but the urinary pH in this dog did not differ from the other infected cases. The bacterial counts were not very high when compared with clinical cases of urolithiasis and perhaps the urease produced was insufficient to alter the pH.

5. CONCLUSIONS

In vitro experiment showed that canine calculi composed of magnesium ammonium phosphate will slowly dissolve in normal dog urine.

Staphylococci multiplied readily in canine urine but *Proteus* organisms failed to survive in this medium.

In vitro infection of canine urine with staphylococci and *Proteus* produced a marked rise in pH and a considerable deposition of magnesium ammonium phosphate crystals. Calculi fragments placed in such urine did not greatly change and no new calculi were formed.

Although under experimental conditions bacterial infection produced precipitation of magnesium ammonium phosphate crystals, factors other than the presence of infection in urine appear to be necessary for the formation of calculi.

In vivo experimental infection of three dogs with staphylococci resulted in a magnesium ammonium phosphate calculus being formed in one animal within 7 months but no calculi were found in the other two dogs where infection had persisted for 12 months. In three dogs, similarly treated but without infection, no calculi developed in 12 months.

The pH of urine from the dogs experimentally infected with staphylococci, although raised, was not significantly different from the urinary pH of the uninfected animals.

GENERAL CONCLUSIONS

In the clinical cases studied the most frequently occurring calculi were composed of magnesium ammonium phosphate and with these calculi the presence of urinary staphylococcal infection was particularly significant. Other types of calculi had a low incidence of infection.

Histological examination of the organic matrices of magnesium ammonium phosphate calculi showed that a very high proportion of them contained cocci.

When magnesium ammonium phosphate calculi recurred they were usually associated with staphylococcal infection but the recurrence rate of this type of stone was significantly less than the recurrence rates for calcium and cystine calculi. It is possible that the treatment which controlled infection is partly responsible for the reduced recurrence of magnesium ammonium phosphate stones.

Dogs with urinary infection show an increased blood leucocyte count and urinary obstruction produces a further increase.

Deposits of magnesium ammonium phosphate crystals also occur in the urinary tracts of cats and sheep but the present study showed that they are not associated with bacterial infection.

Study of the calculi removed from dogs showed that magnesium ammonium phosphate calculi were generally larger than the other types of stones. The number, size, shape and surface texture of the calculi appeared to be related and the shape and surface texture were also related to the shape of the organ in which the calculi developed.

The internal structure of canine calculi contained many of the features described in calculi of human origin. Nuclei were present in a minority of the stones. Many of the calculi were composed of alternating bands of crystals

of differing composition but the reason for this formation was not determined. Fissures were found in the centre of several magnesium ammonium phosphate calculi.

It was confirmed that the staphylococci isolated from dogs with magnesium ammonium phosphate urolithiasis were usually able to produce urease, coagulase and phosphatase and to haemolyse sheep blood. The action of urease and phosphatase on urine could reduce the solubility of magnesium ammonium phosphate and promote crystallization but as these enzymes were not invariably present their influence is not essential to calculus formation.

The in vitro experiments on calculus growth in canine urine showed that calculi slowly dissolved in uninfected urine. When calculi were placed in urine infected with staphylococci or Proteus organisms little change occurred to the calculi but there was a heavy deposit of magnesium ammonium phosphate crystals from the urine. These findings indicate that bacteria can cause precipitation of crystals from urine but under the experimental conditions they did not cause significant changes in the calculi.

Attempts to reproduce urolithiasis in three dogs by establishing bladder infection with staphylococci produced inconclusive results. One dog developed a magnesium ammonium phosphate calculus but in two others no calculi were formed.

The present study establishes further evidence in support of the association between staphylococcal infection and magnesium ammonium phosphate urolithiasis. This evidence is of clinical significance because the successful treatment of these calculi depends on the elimination of staphylococci from the urinary tract. As all the circumstances by which staphylococci may become established in the bladder are not known and therefore cannot be anticipated, the prevention of magnesium ammonium phosphate urolithiasis does not yet seem to be practicable.

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REFERENCES

American Society for Testing Materials (1959) "Index to the X-ray Powder Data File", Philadelphia: American Society for Testing Materials.

Asscher, A.W., Sussman, M., Waters, W.E., Davis, R.H. and Chick, S. (1966) Lancet, 2, 1037.

Barber, M., Brooksbank, B.W.L. and Kuper, S.W.A. (1951) J. Path. Bact., 63, 57.

Barber, M. and Kuper, S.W.A. (1951) J. Path. Bact., 63, 65.

Barnhouse, D.H. (1968) Invest. Urol., 5, 342.

Benjamin, J.A., Wilson, J.G. and Leahy, A.D. (1945) J. Urol., 54, 516.

Beveridge, W.I.B. (1942) Aust. vet. J., 18, 127.

Bloom, F. (1954) "Pathology of the Dog and Cat", Evanston, Illinois: American Veterinary Publications.

Blount, W.P. (1931) Vet. J., 87, 561.

Boivin, A. and Mesrobeanu, L. (1933) C. r. Séanc. Soc. Biol., 112, 611.

Boyce, W.H. and Garvey, F.K. (1956) J. Urol., 76, 213.

Boyce, W.H. and Sulkin, N.M. (1956) J. clin. Invest., 35, 1067.

Boyce, W.H., Pool, C.S., Meschan, I. and King, J.S. (1958) Acta radiol., 50, 543.

Boyce, W.H. and King, J.S. (1959) J. Urol., 81, 351.

Boyce, W.H. (1969) In "Renal Stone Research Symposium", 93, edited by Hodgkinson, A. and Nordin, B.E.C., London: Churchill.

Brand, E., Cahill, G.F. and Kassell, B. (1940) J. biol. Chem., 133, 431.

Braude, A.I. and Siemienski, J. (1960) J. Bact., 80, 171.

Bray, J. and King, E.J. (1942) J. Path. Bact., 54, 287.

Bray, J. and King, E.J. (1943) J. Path. Bact., 55, 315.

Brodey, R.S. (1955) J. Am. vet. med. Ass., 126, 1.

Brown, T.R. (1901) J. Am. med. Ass., 36, 1395.

Bugbee, H.G. (1932) Trans. Am. Ass. genito-urin. Surg., 25, 121.

Carbone, M.G. (1965) J. Am. vet. med. Ass., 147, 1195.

Carr, J.A. (1953) Br. J. Urol., 25, 26.

Carroll, G. and Brennan, R.V. (1952a) J. int. Coll. Surg., 17, 809.

Carroll, G. and Brennan, R.V. (1952b) J. Urol., 68, 88.

Christensen, W.B. (1946) J. Bact., 52, 461.

Chute, R. (1938) New Engl. J. Med., 219, 1030.

Clark, S.M. and Iball, J. (1957) "Progress in Biophysics and Biophysical Chemistry", 7, London: Pergamon Press.

Collica, I. (1948) Am. J. Surg., 76, 424.

Cordonnier, J.J. and Millar, J.A. (1951) J. Urol., 66, 12.

Cornelius, C.E. and Bishop, J.A. (1961) J. Urol., 85, 842.

Cosslett, V.E. and Nixon, W.C. (1960) "X-ray Microscopy", Cambridge: Cambridge University Press.

Cotran, R.S. (1963) "Angiotensin Systems and Experimental Renal Diseases", 132, edited by Metcoff, J., London: Churchill.

Cowan, S.T. and Steel, K.J. (1965) "Manual for the Identification of Medical Bacteria", Cambridge: Cambridge University Press.

Cox, C.E. and Hinman, F. (1961) J. Urol., 86, 739.

Crabtree, E.G. (1943) J. Urol, 49, 652.

Cruikshank, R. (1965) "Medical Microbiology", 11th ed., Edinburgh: Livingstone.

Davalos, H.A. (1943) J. Urol., 49, 639.

Davis, E.G. and Hain, R.F. (1918) J. Urol., 2, 309.

Day, A.A., Gibbs, W.M., Walker, A.W. and Jung, R.E. (1930) J. infect. Dis., 47, 490.

Douglas, S.W. and Williamson, H.D. (1963) "Principles of Veterinary Radiography", London: Baillière, Tindall and Cox.

Doxey, D.L. (1964) "The Extent of Variations in the Blood Picture of Normal Dogs and the Deviations Encountered in some Canine Diseases", Ph.D. Thesis, Edinburgh University.

Earlam, M.S.S. (1930) Br. J. Urol., 2, 233.

Edwards, C.N., Garvey, F.K. and Boyce, W.H. (1963) J. Urol., 89, 207.

Eisenstaedt, J.S. (1931) Surgery Gynec. Obstet., 53, 730.

Elek, S.D. and Levy, E. (1950) J. Path. Bact., 62, 541.

Elliot, J.S., Quaide, W.L., Sharp, R.F. and Lewis, L. (1958) J. Urol., 80, 269.

Elliot, J.S., Sharp, R.F. and Lewis, L. (1959) J. Urol., 81, 366.

Elliot, J.S. (1968) J. Urol., 100, 687.

Engström, A. (1946) Acta radiol., Supplement 63.

Finco, D.R., Kurtz, H.J. and Porter, T.E. (1970) J. Am. vet. med. Ass., 157, 840.

Finco, D.R., Rosin, E. and Johnson, K.H. (1970) J. Am. vet. med. Ass., 157, 1225.

Fisher, R.A. (1970) "Statistical Methods for Research Workers", 14th ed., Edinburgh: Oliver and Boyd.

Fishler, J. (1955) J. Am. vet. med. Ass., 127, 121.

Foster, S.J. (1967) J. small Anim. Pract., 8, 207.

Friedman, S.A. and Gladstone, J.L.(1971) J. Urol., 105, 428.

Fritsch, R. and Zuylen, A.L. (1966) Tierarztl. Umsch., 21, 551.

Frost, R.C. (1958) Vet. Rec., 70, 765.

Geigy Scientific Tables (1970) 7th ed., Basle: Geigy.

Giertz, G. (1946) Acta chir. scand., 94, Supplement 109.

Gordon, J. and Marshall, P.G. (1930) Br. J. exp. Path., 11, 173.

Gordon, J. and Cooper, K.E. (1932) Br. J. exp. Path., 13, 503.

Goulden, B.E. (1966) "A Clinical Study of Canine Urolithiasis", Ph.D. Thesis, Edinburgh University.

Goulden, B.E. (1968) Vet. Rec., 83, 509.

Goulden, B.E. (1969) N.Z. vet. J., 17, 57.

Gregory, J.G., Wein, A.J., Sansone, T.C. and Murphy, J.J. (1971) J. Urol., 105, 220.

Hain, R.F. (1920) J. Urol., 4, 177.

Haugh, I., Lonsdale, K., Mason, P. and White, E.G. (1966) J. small Anim. Pract., 7, 565.

Hedenberg, I., Engfelt, B. and Engström, A. (1953) Br. J. Urol., 25, 33.

Hedenberg, I. (1954) Acta chir. scand., Supplement 192.

Hellström, J. (1936) Acta chir. scand., 79, Supplement 46.

Hellström, J. (1956) In "Etiologic Factors in Urolithiasis", 212, edited by Butt, A.J., Springfield: Thomas.

Higgins, C.C. (1939) J. Am. med. Ass., 113, 1460.

Hill, A.B. (1966) "Principles of Medical Statistics", 8th ed., London: Lancet Ltd.

Hill, J.H. and White, E.C. (1941) J. Urol., 45, 749.

Hobday, F. (1922) Vet. Rec., 2, 519.

Hodgkinson, A., Peacock, M. and Nicholson, M. (1969) Invest. Urol., 6, 549.

Hryntschak, T. (1934) Surgery Gynec. Obstet., 58, 103.

Hueper, W.C., Fisher, C.V., Carvajal-Forero, J. and Thompson, M.R. (1941) J. Urol., 45, 186.

Johnson, D.W., Palmer, L.S. and Nelson, J.W. (1940) Vet. Med., 35, 353.

Jolly, J.S. (1929) "Stone and Calculous Disease of the Urinary Organs", London: Heinemann.

Keyser, L.D. (1923) Archs Surg., 6, 525.

Keyser, L.D. (1934) J. Urol., 31, 219.

King, J.S. and Boyce, W.H. (1957) Proc. Soc. exp. Biol. Med., 95, 183.

King, J.S. and Boyce, W.H. (1963) J. Urol., 89, 546.

Krabbe, A. (1949) Vet. Rec., 61, 759.

Lagergren, C. (1956) Acta radiol., Supplement 133.

Lauder, J. (1949) Vet. Rec., 61, 757.

Lonsdale, K. (1968a) Science, N.Y., 159, 1199.

Lonsdale, K. (1968b) Nature, Lond., 217, 56.

Lonsdale, K., Sutor, D.J. and Wooley, S. (1968) Br. J. Urol., 40, 33.

Lyon, E.S. and Vermeulen, C.W. (1966) Invest. Urol., 3, 309.

McCarrison, R. (1926) Indian J. med. Res., 14, 895.

McCunn, J. (1934) Vet. Rec., 14, 599.

McCunn, J. (1947) "Hobday's Surgical Diseases of the Dog and Cat", 5th ed., London: Baillière, Tindall and Cox.

MacIntosh, J.F. (1942) J. clin. Invest., 21, 755.

Magens, H.J. (1934) N. Am. Vet., 15, 28.

Mani, P. and Scott, F.B. (1967) Sth. med. J., 60, 1177.

Maurice, P.F. and Henneman, R.H. (1961) Medicine, Baltimore, 40, 315.

Meier, F.W. (1967) J. Am. vet. med. Ass., 151, 1059.

Milks, H.J. (1935) Cornell Vet., 25, 153.

Morton, W.J.T. (1844) "On Calculous Concretions in the Horse, Ox, Sheep and Dog", London: Longman, Brown, Green and Longman.

Mosier, J.E. (1965) Proceedings of the American Animal Hospital Association for 1965, 80.

Murphy, B.T. and Pyrah, L.N. (1962) Br. J. Urol., 34, 129.

Newcomb, C. and Ranganathen, S. (1929) Indian J. med. Res., 17, 1055.

Newsom, I.E. (1938) J. Am. vet. med. Ass., 92, 495.

Nielsen, I.M. (1956) J. Urol., 75, 602.

Norden, C.W., Green, G.M. and Kass, E.H. (1968) J. clin. Invest., 47, 2689.

Ord, W.M. and Shattock, S.G. (1895) Trans. path. Soc., Lond., 46, 91.

Orstadius, K. and Dahlberg, G. (1966) Nord VetMed., 18, 497.

Osborne, T.B., Mendel, L.B. and Ferry, E.L. (1917) J. Am. med. Ass., 69, 32.

Oser, B.L. (1965) "Hawk's Physiological Chemistry", 14th ed., London: McGraw-Hill.

Pasteur, L. and Joubert, J. (1876) C.r. Seanc. Acad. Sci., 83, 5.

Piermattei, D.L. (1960) N.Y. City Vet., April, 1960, 6.

Pillet, P. (1927) J. Urol. méd. chir., 24, 5.

Prien, E.L. and Frondel, C. (1947) J. Urol., 57, 949.

Prien, E.L. (1949) J. Urol., 61, 821.

Prien, E.L. (1955) J. Urol., 73, 627.

Priestley, J.T. and Osterberg, A.E. (1936) J. Urol., 36, 447.

Prout, W. (1848) "On the Nature and Treatment of Stomach and Renal Diseases", 5th ed., London: Churchill.

Rich, L.J. and Kirk, R.W. (1968) Am. J. vet. Res., 29, 2149.

Rich, L.J. and Kirk, R.W. (1969) J. Am. vet. med. Ass., 154, 153.

Robertson, W.G. and Nordin, B.E.C. (1969) In "Renal Stone Research Symposium", 221, edited by Hodgkinson, A. and Nordin, B.E.C., London: Churchill.

Rovsing, C.M. (1924) Acta chir. scand., 57, 387.

Schalm, O.W. (1965) "Veterinary Haematology", 2nd ed., London: Baillière, Tindall and Cassell.

Schechter, R.D. (1970) J. Am. vet. med. Ass., 156, 1567.

Schoenberg, H.W., Beisswanger, P., Howard, W.J., Walter, C.F. and Murphy, J.J. (1965) In "Progress in Pyelonephritis", 632, edited by Kass, E.H., Philadelphia: Davis.

Schulte, T.L. and Thompson, G.J. (1941) J. Urol., 45, 733.

Scott, J.E.S. (1964) Br. J. Urol., 36, 501.

Smale, D.E. (1956) Br. dent. J., 100, 244.

Smith, G.G. (1939) Surgery Gynec. Obstet., 68, 527.

Smith, H.W. (1947) J. comp. Path. Ther., 57, 98.

Smith, H.A. and Jones, T.C. (1966) "Veterinary Pathology", 3rd ed., London: Baillière, Tindall and Cassell.

Smith, S.E. and Hodson, A.Z. (1941) Cornell Vet., 31, 30.

Snedecor, G.W. (1956) "Statistical Methods", 5th ed., Ames, Iowa: Iowa State College Press.

Sommer, J.L. and Roberts, J.A. (1966) J. Urol., 95, 502.

Sompolinsky, D. (1947) Maanedsskr. Dyrlaeg., 58, 401.

Stainton, F.H. (1922) Vet. Rec., 2, 347.

Stockman, V. (1972) J. small Anim. Pract., 13, 635.

Suby, H.I. and Suby, R.M. (1947) J. Urol., 57, 995.

Sunshine, H. (1964) J. Urol., 92, 351.

Sutor, D.J. (1968) Br. J. Urol., 40, 29.

Sutor, D.J. and Scheidt, S. (1968) Br. J. Urol., 40, 22.

Sutor, D.J. and Wooley, S.E. (1970) Res. vet. Sci., 11, 299.

Sutor, D.J., Wooley, S.E. and Jackson, O.F. (1970) Res. vet. Sci., 11, 298.

Thomas, W.C.,Bird, E.D. and Tomita, E. (1963) J. Urol., 90, 521.

Thompson, H.E., Steadman, L.T., Benjamin, J.A. and Scott, W.W. (1944) J. Urol., 51, 259.

Treacher, R.J. (1966) J. small Anim. Pract., 7, 537.

Trueman, N.A. and Stacy, B.D. (1969) Invest. Urol., 7, 185.

Udall, R.H. and Chow, F.H.C. (1963) Ann. N.Y. Acad. Sci., 104, 612.

United States Department of Commerce National Bureau of Standards (1950) "Tables for Conversion of X-ray Diffraction Angles to Interplaner Spacing," Washington: United States Department of Commerce.

Van Slyke, D.D. and Archibald, R.M. (1944) J. biol. Chem., 154, 623.

Vejlsgaard, R. (1965) In "Progress in Pyelonephritis", 468, edited by Kass, E.H., Philadelphia: Davis.

Vermeulen, C.W., Grove, W.J., Goetz, R., Ragins, H.D. and Correll, N.O. (1950) J. Urol., 64, 541.

Vermeulen, C.W., Ragins, H.D., Grove, W.J. and Goetz, R. (1951) J. Urol, 66, 1.

Vermeulen, C.W., Lyon, E.S. and Gill, W.B. (1964) Invest. Urol., 1, 370.

Vermeulen, C.W., Ellis, J.E. and Hsu, T.C. (1966) J. Urol., 95, 681.

Weaver, A.D. (1970) J. small Anim. Pract., 11, 93.

White, A., Handler, P. and Smith, E.L. (1959) "Principles of Biochemistry", 3rd ed., London: McGraw-Hill.

White, E.C. and Hill, J.H. (1941) J. Urol., 45, 744.

White, E.G. (1966) J. small Anim. Pract., 7, 529.

White, E.G., Treacher, R.J. and Porter, P. (1961) J. comp. Path. Ther., 71, 201.

Williams, R.E. (1969) In "Renal Stone Research Symposium", 65, edited by Hodgkinson, A. and Nordin, B.E.C., London: Churchill.

Wilson, J.G., Benjamin, J.A. and Leahy, A.D. (1945) J. Urol., 54, 503.

Yudofsky, S.C. and Scott, F.B. (1969) J. Urol., 102, 745.

Young, H.H. and Davis, D.M. (1926) "Young's Practice of Urology", Philadelphia: Saunders.